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<b>(21) International Application Number:</b> PCT/US00/13694  <b>(22) International Filing Date:</b> 18 May 2000 (18.05.00)  <b>(30) Priority Data:</b> 60/134,702      18 May 1999 (18.05.99)      US  <b>(71) Applicant (for all designated States except US):</b> SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> GROSS, Mitchell, S. [US/US]; 667 Pugh Road, Wayne, PA 19087 (US). SWEET, Raymond, W. [US/US]; 108 Edgehill Road, Bala Cynwyd, PA 19004 (US). TAYLOR, Geraldine [GB/GB]; Compton, Newbury, Berkshire RG20 7NN (GB).  <b>(74) Agents:</b> BAUMEISTER, Kirk et al.; Smithkline Beecham Corporation, Corporate Intellectual Property, U/W2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).		<b>(81) Designated States:</b> AE, AL, AU, BA, BB, BG, BR, CA, CN, CZ, DZ, EE, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MA, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, TZ, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> HUMAN MONOCLONAL ANTIBODY  <b>(57) Abstract</b>  This invention relates to novel human monoclonal antibodies (mAbs) and to the genes encoding same. More specifically, this invention relates to human monoclonal antibodies specifically reactive with an epitope of the fusion (F) protein of Respiratory Syncytial Virus (RSV). Such antibodies are useful for the therapeutic and/or prophylactic treatment of RSV infection in human patients, particularly infants and young children.		

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## HUMAN MONOCLONAL ANTIBODY

**Field of the Invention**

5        This invention relates to novel human monoclonal antibodies (mAbs) and to the genes encoding same. More specifically, this invention relates to human monoclonal antibodies specifically reactive with an epitope of the fusion (F) protein of Respiratory Syncytial Virus (RSV).  
10      Such antibodies are useful for the therapeutic and/or prophylactic treatment of RSV infection in human patients, particularly infants and young children.

**Background of the Invention**

15        Respiratory syncytial virus (RSV) is the major cause of lower respiratory disease in children, giving rise to predictable annual epidemics of bronchiolitis and pneumonia in children worldwide. The virus is highly contagious, and infections can occur at any age.  
20      Comprehensive details concerning RSV infection and its clinical features can be obtained from excellent recent reviews by McIntosh, K. and R. M. Chanock, In: "Respiratory Syncytial Virus", Ch. 38, B.N. Fields ed., Raven Press (1990) and Hall, C.B., In: "Textbook of  
25      Pediatric Disease" Feigin and Cherry, eds., W.B. Saunders, pgs 1247-1268 (1987).

      RSV is distributed worldwide. One of the most remarkable features of the epidemiology of RSV virus, as mentioned above, is the consistent pattern of infection  
30      and disease. Other respiratory viruses cause epidemics at irregular intervals or exhibit a mixed endemic/epidemic pattern, but RSV is the only respiratory viral pathogen that produces a sizable epidemic every year in large urban centers. In the

temperate areas of the world, RSV epidemics have occurred primarily in the late fall, winter or spring but never during the summer. The occurrence and spread of infection within a community is characteristic and easily diagnosed, leading to sharp rises in cases of bronchiolitis and pediatric pneumonia and the number of hospital admissions of young children with acute lower respiratory tract disease. Other respiratory viral agents that occur in outbreaks are rarely present at the same time as RSV.

Primary RSV infection occurs in the very young. Zero to 2 year old infants are the most susceptible and represent the primary affected population. In this group, 1 out of 5 will develop lower respiratory (below larynx) disease upon infection and this ratio stays the same upon reinfection. By 1 year of age, 25-50% of infants have specific antibodies as a result of natural infection and this is close to 100% by age 4-5. Thus, virtually all children have been infected before they have entered school.

Age, sex, socioeconomic and environmental factors can all influence the severity of disease. Hospitalization is required in 1-3% of cases of RSV infection and is usually of long duration (up to 3 weeks). The high morbidity of RSV infection, especially in infancy, has also been implicated in the development of respiratory problems later in life. With current intensive care in the U.S. and the other developed countries, overall mortality for normal subjects is low (less than 2% of hospitalized subjects). However, mortality is much higher in less developed countries and, even in developed countries, mortality is high in certain risk groups such as in infants with underlying cardiac condition (cyanotic congenital heart disease) or

respiratory disease (bronchopulmonary dysplasia) where the progression of symptoms may be rapid. For instance, mortality in infants with cyanotic congenital heart disease has been reported to be as high as 37%. In  
5 premature infants apneic spells due to RSV infection may occur and, in rare cases, cause neurologic or systemic damage. Severe lower respiratory tract illness (bronchiolitis and pneumonia) is most common in patients under six months of age. Infants who have apparently  
10 recovered completely from this illness may display symptomatic respiratory abnormalities for years (recurrent wheezing, decreased pulmonary function, recurrent cough, asthma, and bronchitis).

Immunity to RSV appears to be short-lived, thus  
15 reinfections are frequent. The mechanisms by which the immune system protects against RSV infection and reinfection are not well understood. It is clear, however, that immunity is only partially protective since reinfection is common at all ages, and sometimes  
20 occurs in infants only weeks after recovery from a primary infection. Both serum and secretory antibodies (IgA) have been detected in response to RSV infection in adults as well as in very young infants. However, the titers of serum antibodies to the viral F or G  
25 glycoprotein, as well as of neutralizing antibodies found in infants (1-8 months of age) are 15-25% of those found in older subjects. These reduced titers may contribute to the increased incidence of serious infection in younger children.

30 Evidence for the role of serum antibodies in protection against RSV virus has emerged from epidemiological as well as animal studies. In adults exposed naturally to the virus, susceptibility correlated well with low serum antibody level. In

infants, titers of maternally transmitted antibodies correlate with resistance to serious disease [Glezen, W.P. et al., J. Pediatr. 98:708-715 (1981)]. Other studies show that the incidence and severity of lower  
5 respiratory tract involvement is diminished in the presence of high serum antibody [McIntosh, K. et al., J. Infect. Dis. 138:24-32 (1978)] and high titers of passively administered serum neutralizing antibodies have been shown to be protective in a cotton rat model  
10 of RSV infection [Prince, G. A. et al., Virus Res. 3:193-206 (1985)].

Children lacking cell-mediated immunity are unable to overcome their infection and shed virus for many months in contrast to children with normal immune  
15 systems. Similarly, nude mice infected with RSV virus persistently shed virus. These mice can be cured by adoptive transfer of primed T cells [Cannon, M. J. et al., Immunology 62:133-138 (1987)].

In summary, it appears that both cellular and  
20 humoral immunity are involved in protection against infection, reinfection and RSV disease and that although antigenic variation is limited, protective immunity is not complete even after multiple exposures.

RSV, belonging to the family paramyxoviridae, is a  
25 negative-strand unsegmented RNA virus with properties similar to those of the paramyxoviruses. It has, however been placed in a separate genus Pneumovirus, based on morphologic differences and lack of hemagglutinin and neuraminidase activities. RSV is  
30 pleomorphic and ranges in size from 150-300 nm in diameter. The virus matures by budding from the outer membrane of a cell and virions appear as membrane-bound particles with short, closely spaced projections or "spikes". The RNA genome encodes 10 unique viral

polypeptides ranging in size from 9.5 kDa to 160 kDa [Huang, Y. T. and G. W. Wertz, J. Virol. 43:150-157 (1982)]. Seven proteins (F, G, N, P, L, M, M2) are present in RSV virions and at least three proteins (F, G, and SH) are expressed on the surface of infected cells. The F protein [SEQ ID NO: 20] has been conclusively identified as the protein responsible for cell fusion since specific antibodies to this protein inhibit syncytia formation *in vitro* and cells infected with vaccinia virus expressing recombinant F protein form syncytia in the absence of other RSV virus proteins. In contrast, antibodies to the G protein do not block syncytia formation but prevent attachment of the virus to cells.

RSV can be divided into two antigenically distinct subgroups, (A & B) [Mufson, M. A. et al., J. Gen'l. Virol. 66:2111-2124 (1985)]. This antigenic dimorphism is linked primarily to the surface attachment (G) glycoprotein [Johnson, R. A. et al., Proc. Nat'l. Acad. Sci. USA 84:5625-5629 (1987)]. Strains of both group A and B circulate simultaneously, but the proportion of each may vary unpredictably from year to year. An effective therapy must therefore target both subgroups of the virus and this is the reason for the selection of the highly conserved surface fusion (F) protein as target antigen for mAb therapy as will be discussed later.

The induction of neutralizing antibodies to RSV virus appears to be limited to the F and G surface glycoproteins. Of these two proteins, the F protein is the major target for cross-reactive neutralizing antibodies associated with protection against different strains of RSV virus. In addition, experimental vaccination of mice or cotton rats with F protein also

results in cross protection. The antigenic relatedness of the F protein across strains and subgroups of the virus is reflected in its high degree of homology at the amino acid level. In contrast, in the two subgroups and various strains of RSV, antigenic dimorphism was linked primarily to the G glycoprotein. The F protein has a predicted molecular weight of 68-70 kDa; a signal peptide at its N-terminus; a membrane anchor domain at its C terminus; and is cleaved proteolytically in the infected cell prior to virion assembly to yield disulfide linked F<sub>2</sub> and F<sub>1</sub>. Five neutralizing epitopes have been identified within the F protein sequence [SEQ ID NO: 20] and map to residues 205-225; 259-278; 289-299; 483-488 and 417-438. Studies to determine the frequency of sequence diversion in the F protein [SEQ ID NO: 20] showed that the majority of the neutralizing epitopes were conserved in all of the 23 strains of RSV virus isolated in Australia, Europe, and regions of the U.S. over a period of thirty years. In another study, seroresponses of forty three infants and young children to primary infection with subgroup A or a subgroup B strain showed that responses to homologous and heterologous F antigens were not significantly different, while the G proteins of the subgroup A and B strains were quite unrelated. Moreover, antibody inhibition of virus-mediated cell fusion *in vitro* versus inhibition of infection correlates best with protection in animal models and fusion inhibition is primarily restricted to F protein specific antibodies.

Prophylactic treatment for RSV infection is thus desirable for the high risk groups of children as well as for all children in underdeveloped countries. However, a vaccine for RSV infection is not currently available. Severe safety issues surrounding an



attenuated whole virus vaccine tested in the 1960s, as well as the potential of induced immunopathology associated with the newer candidate subunit vaccines make the prospects of a vaccine in the near future appear remote. To date one drug therapy, Ribavirin, a broad spectrum antiviral, has been approved. Ribavirin has gained only minimal acceptance owing to problems of administration, mild toxicity and questionable efficacy. In the majority of cases, hospitalized children receive no drug therapy and receive only intensive supportive care which is extremely costly. It is clear that there is a need for a safe, effective and easily administered drug for the treatment of RSV infection.

The use of passive antibody therapy in humans is well documented and is being used to treat other infectious diseases such as hepatitis and cytomegalovirus. The feasibility of passive antibody treatment/protection against RSV has been well established using animal models. Most of the earlier passive transfer studies in animals against infectious agents, including RSV, utilized murine mAbs. Studies in animals have clearly demonstrated that polyclonal and monoclonal antibody against both F and G glycoprotein can confer passive protection in RSV virus infection when given prophylactically or therapeutically [Prince, *et al.*, supra]. In these studies, passive transfer of neutralizing F or G mAbs to mice, cotton rats or monkeys, significantly reduce or completely prevent replication of the RSV virus in the lungs. However, as discussed above, clearly, the F protein is the more important target for antibody therapy.

Recently, the FDA has approved for use intravenous gammaglobulins (IVIG) isolated from pooled human sera. Initial reports from this study had been encouraging

[Groothuis, J. R. *et al.*, Antimicrob. Agents Chemo. 35(7):1469-1473 (1991)]. However, generic shortcomings of IVIGs exist and include, without limitation, the fact that such products are human blood derived and grams of antibody often need to be administered to achieve an effective dose.

Alternatively, monoclonal antibodies have been employed. The advantages of such an approach include: a higher concentration of specific antibody can be achieved thereby reducing the amount of globulin required to be given; the reliance on direct blood products can be eliminated; the levels of antibody in the preparation can be more uniformly controlled and the routes of administration can be extended. While passive immunotherapy employing monoclonal antibodies from a heterologous species (e.g., murine) has been suggested (See: PCT Application PCT/US94/08699, Publication No. WO 95/04081), one alternative to reduce the risk of an undesirable immune response on the part of the patient directed against the foreign antibody is to employ "humanized" antibodies. These antibodies are substantially of human origin, with only the Complementarity Determining Regions (CDRs) being of non-human origin. Particularly useful examples of this approach are disclosed in PCT Application PCT/GB91/01554, Publication No. WO 92/04381 and PCT Application PCT/GB93/00725, Publication No. WO93/20210. Clinical trials are on-going to evaluate the efficacy of humanized antibodies for treatment of RSV infection in young children.

A second and more preferred approach is to employ fully human mAbs. Unfortunately, there have been few successes in producing human monoclonal antibodies through classic hybridoma technology. Indeed,

acceptable human fusion partners have not been identified and murine myeloma fusion partners do not work well with human cells, yielding unstable and low producing hybridoma lines. However, recent advances in molecular biology and immunology make it now possible to isolate human mAbs, particularly directed against foreign infectious agents.

Fully human mAbs to RSV F protein [SEQ ID NO: 20] remain a desirable option for the treatment of this disease. Although some success has been reported in obtaining fragments of such mAbs [Barbas, C.F. et al., Proc. Nat'l. Acad. Sci. USA 89:10164-10168 (1992); Crowe, J. E. et al., Proc. Nat'l. Acad. Sci. USA 91: 1386-1390 (1994) and PCT application number PCT/US93/08786, published as WO94/06448, March 31, 1994)], the achievement of such results is not straightforward. Novel human mAbs, when and however obtained, are particularly useful alone or in combination with existing molecules to form immunotherapeutic compositions.

There exists a need in the art for useful prophylactic compositions for the prevention or passive treatment of RSV.

## **Brief Description of the Invention**

In one aspect, this invention provides fully human monoclonal antibodies and functional fragments thereof specifically reactive with an F protein epitope of RSV and capable of neutralizing RSV infection. These human mAbs specific for the F protein of RSV virus may be useful to passively treat or prevent infection.

In another aspect, the present invention provides modifications to neutralizing single chain Fv fragments (scFv) specific for the F protein of RSV produced by

random combinatorial cloning of human antibody sequences and isolated from a filamentous phage Fab display library.

In still another aspect, there is provided a  
5 reshaped or altered human antibody containing human heavy and light chain constant regions from a first human donor and heavy and light chain variable regions or the CDRs thereof derived from human neutralizing monoclonal antibodies for the F protein of RSV derived  
10 from a second human donor.

In yet another aspect, the present invention provides a pharmaceutical composition which contains one (or more) altered or reshaped antibodies and a pharmaceutically acceptable carrier.

15 In yet another aspect, the invention provides a pharmaceutical composition comprising at least one dose of an immunotherapeutically effective amount of the reshaped, altered or monoclonal antibody of this invention in combination with at least one additional  
20 monoclonal, altered or reshaped antibody. A particular embodiment is provided in which the additional antibody is an anti-RSV antibody distinguished from the subject antibody of the invention by virtue of being reactive with a different epitope of the RSV F protein antigen  
25 than the subject antibody of the invention.

In a further aspect, the present invention provides a method for passive immunotherapy of RSV disease in a human by administering to said human an effective amount of the pharmaceutical composition of the invention for  
30 the prophylactic or therapeutic treatment of RSV infection.

In yet another aspect, the present invention provides methods for, and components useful in, the recombinant production of human and altered antibodies

(e.g., engineered antibodies, CDRs, Fab or F(ab)<sub>2</sub> fragments, or analogs thereof) which are derived from human neutralizing monoclonal antibodies (mAbs) for the F protein of RSV. These components include isolated  
5 nucleic acid sequences encoding same, recombinant plasmids containing the nucleic acid sequences under the control of selected regulatory sequences which are capable of directing the expression thereof in host cells (preferably mammalian) transfected with the  
10 recombinant plasmids. The production method involves culturing a transfected host cell line of the present invention under conditions such that the human or altered antibody is expressed in said cells and isolating the expressed product therefrom.

15 In still another aspect of the invention is a method to diagnose the presence of RSV in a human which comprises contacting a sample of biological fluid with the human antibodies and altered antibodies and fragments thereof of the instant invention and assaying  
20 for the occurrence of binding between said human antibody (or altered antibody, or fragment) and RSV.

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

25

#### **Brief Description of the Drawings**

Fig. 1A is a graph illustrating the competition of Gλ-1 scFV phage binding with RSV19 mAb [International patent publication No. WO92/04381, published March 19,  
30 1992].

Fig. 1B is a graph illustrating the competition of Gλ-1 scFV phage binding with RSV B4 mAb [International patent publication No. WO93/20210, published October 14, 1993].

Fig. 2 is a graph illustrating virus neutralization by scFV phages, G $\lambda$ -1, G $\lambda$ -3, and G $\kappa$ -1 with RSV strain 273.

Fig. 3 illustrates the DNA sequence [SEQ ID NO: 1] and protein sequence (amino acids reported in single letter code) [SEQ ID NO: 2] for the G $\lambda$ -1 light chain variable region, processed N-terminus through framework IV.

Fig. 4 illustrates the DNA sequence [SEQ ID NO: 3] and protein sequence (amino acids reported in single letter code) [SEQ ID NO: 4] for the G $\lambda$ -1 heavy chain variable region, processed N-terminus through framework IV.

Fig. 5 illustrates the cloning strategy used for the construction of the G $\lambda$ -1 monoclonal antibody. The heavy chain V region was cloned into the pCD derivative vector as a XhoI - ApaI fragment. The entire light chain V region was cloned into the pCN derivative vector, 43-lpcn, as a SacI - AvrII fragment. Details are described below.

Fig. 6 provides a comparison of the heavy chain amino acid sequences of the G $\lambda$ -1 single chain F $_v$  [SEQ ID NO: 5] and various monoclonal antibodies of this invention. The amino acid sequences of the heavy chains for the A [SEQ ID NO: 7] and B [SEQ ID NO: 8] constructs are shown. Numbering of the residues is based on the germline (GL) gene Dp58 [SEQ ID NO: 6], beginning at the mature processed amino terminus and ending at CDR3. The "-" indicates identity to the preceding sequence (eg., A compared to B). Bold residues correspond to the leader region, and to CDRs 1-3.

Fig. 7 provides a comparison of the light chain amino acid sequences of the G $\lambda$ -1A single chain F $_v$  [SEQ ID NO: 9] and various monoclonal antibodies of this

invention. The amino acid sequences of the light chains for the A [SEQ ID NO: 11] and B [SEQ ID NO: 12] constructs are shown. Numbering of the residues in the Vk region is based on the germline (GL) gene DpL8 [SEQ ID NO: 10], beginning at the mature processed amino terminus and ending at CDR3. For reference to framework 4, the actual numbering is also shown for G $\lambda$ -1A. As in Fig. 6, the "-" indicates identity to the preceding sequence.

10 Figs. 8A to 8F illustrate the continuous DNA sequence [SEQ ID NO: 13] of the expression plasmid G $\lambda$ -1Apcd containing the RSV neutralizing human G $\lambda$ -1 mAb for the heavy chain. The start of translation, leader peptide, amino-terminal processing site, carboxy  
15 terminus of the G $\lambda$ -1 heavy chain, and Eco RI restriction endonuclease cleavage site are shown.

Figs. 9A to 9E illustrate the continuous DNA sequence [SEQ ID NO: 14] of the expression plasmid G $\lambda$ -1Apcn containing the RSV neutralizing human G $\lambda$ -1 mAb for  
20 the light chain. The corresponding features for the light chain as for Figs. 8A-8F are shown.

Figs. 10A and 10B illustrate the continuous DNA sequence [SEQ ID NO: 15] of the coding region of the heavy chain of plasmid G $\lambda$ -1Bpcd. Bolded residues  
25 indicate differences from the full vector sequence for G $\lambda$ -1Apcd in Figs. 8A-8F [SEQ ID NO: 13].

Fig. 11 is the DNA sequence [SEQ ID NO: 16] of the coding region for the light chain of plasmid G $\lambda$ -1Bpcn. Bolded residues indicate differences from the full  
30 vector sequence for G $\lambda$ -1Apcn in Figs. 9A-9E [SEQ ID NO: 14].

**Detailed Description of the Invention**

This invention provides useful human monoclonal antibodies (and fragments thereof) reactive with the F protein of RSV, isolated nucleic acids encoding same and various means for their recombinant production as well as therapeutic, prophylactic and diagnostic uses of such antibodies and fragments thereof.

*I. Definitions.*

As used in this specification and the claims, the following terms are defined as follows:

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such altered antibodies are engineered antibodies (e.g., chimeric, humanized, or reshaped or immunologically edited human antibodies) or fragments thereof lacking all or part of an immunoglobulin constant region, e.g., Fv, Fab, or F(ab')<sub>2</sub> and the like.

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding an altered antibody of the invention or a fragment thereof.

"Reshaped human antibody" refers to an altered antibody in which minimally at least one CDR from a first human monoclonal donor antibody is substituted for a CDR in a second human acceptor antibody. Preferably all six CDRs are replaced. More preferably an entire antigen combining region (e.g., Fv, Fab or F(ab')<sub>2</sub>) from a first human donor monoclonal antibody is substituted for the corresponding region in a second human acceptor monoclonal antibody. Most preferably the Fab region from a first human donor is operatively linked to the appropriate constant regions of a second human acceptor antibody to form a full length monoclonal antibody.



"First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor human antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example, Kabat et al. (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)) disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

"Second fusion partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in frame or by means of an optional conventional linker sequence (i.e., operatively linked). Preferably the fusion partner is an immunoglobulin gene and when so, it is referred to as a "second immunoglobulin partner". The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (i.e., homologous - the first and second altered antibodies are derived from the same source) or an additional (i.e., heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found

in a Fab, or F(ab)<sub>2</sub> (i.e., a discrete part of an appropriate human constant region or framework region).

A second fusion partner may also comprise a sequence encoding an integral membrane protein exposed on the  
5 outer surface of a host cell, e.g., as part of a phage display library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase (HRP),  $\beta$ -galactosidase, etc.

The terms Fv, Fc, Fd, Fab, or F(ab')<sub>2</sub> are used with  
10 their standard meanings [see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)].

As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length  
15 synthetic antibody (e.g., a chimeric, humanized, reshaped or immunologically edited human antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous  
20 parts from one or more donor antibodies which have specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa.  
25 Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise  
30 replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains naturally-occurring

variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody from a heterologous species.

5           A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity [see, e.g., Queen et al., 10           Proc. Nat'l. Acad. Sci. USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)].

          An "immunologically edited antibody" refers to a 15           type of engineered antibody in which changes are made in donor and/or acceptor sequences to edit regions in respect of cloning artifacts, germ line enhancements, etc. aimed at reducing the likelihood of an immunological response to the antibody on the part of a 20           patient being treated with the edited antibody.

          The term "donor antibody" refers to an antibody (monoclonal, or recombinant) which contributes the nucleic acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first 25           immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody. One donor antibody suitable for use in this 30           invention is a Fab fragment of a human neutralizing monoclonal antibody designated as Fab Gλ-1. Fab Gλ-1 is defined as a having the variable light and heavy chain DNA and amino acid sequences Gλ-1 as shown in Figs. 3, 4, 8A-8F and 9A-9E [SEQ ID NOS: 1-4, 13 and 14].

The term "acceptor antibody" refers to an antibody (monoclonal or recombinant) from a source genetically unrelated to the donor antibody, which contributes all (or any portion, but preferably all) of the nucleic acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. Preferably a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains [see, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)]. There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

By "sharing the antigen binding specificity or neutralizing ability" is meant, for example, that although Fab G $\lambda$ -1 may be characterized by a certain level of antigen affinity, a CDR encoded by a nucleic acid sequence of Fab G $\lambda$ -1 in an appropriate structural environment may have a lower, or higher affinity. It is

expected that CDRs of Fab G $\lambda$ -1 in such environments will nevertheless recognize the same epitope(s) as does the intact Fab G $\lambda$ -1. A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor  
5 deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or neutralizing ability as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence modified by  
10 at least one amino acid, wherein said modification can be a chemical modification, or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen  
15 specificity and high affinity, of the unmodified sequence. For example, (silent) mutations can be constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions.

20 Analogs may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid or peptide sequences of the invention. Such variations or modifications may be due to degeneracy in the genetic  
25 code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein  
30 carrier molecules to which the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used

in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIAcore (Pharmacia) system, or other non-protein substances useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol.

10        *II. Combinatorial Cloning.*

As mentioned above, a number of problems have hampered the direct application of the hybridoma technology [G. Kohler and C. Milstein, Nature, 256: 495-497 (1975)] to the generation and isolation of human monoclonal antibodies. Among these are a lack of suitable fusion partner myeloma cell lines used to form hybridoma cell lines as well as the poor stability of such hybridomas even when formed. These shortcomings are further exacerbated in the case of RSV because of the paucity of viral specific B cells in the peripheral circulation. Therefore, the molecular biological approach of combinatorial cloning is preferred.

Combinatorial cloning is disclosed generally in PCT Publication No. WO90/14430. Simply stated, the goal of combinatorial cloning is to transfer to a population of bacterial cells the immunological genetic capacity of a human cell, tissue or organ. It is preferred to employ cells, tissues or organs which are immunocompetent. Particularly useful sources include, without limitation, spleen, thymus, lymph nodes, bone marrow, tonsil and peripheral blood lymphocytes. The cells may be optionally RSV stimulated *in vitro*, or selected from donors which are known to have produced an immune response or donors who are HIV<sup>+</sup> but asymptomatic.

The genetic information isolated from the donor cells can be in the form of DNA or RNA and is conveniently amplified by Polymerase Chain Reaction (PCR) or similar techniques. When isolated as RNA the genetic information is preferably converted into cDNA by reverse transcription prior to amplification. The amplification can be generalized or more specifically tailored. For example, by a careful selection of PCR primer sequences, selective amplification of immunoglobulin genes or subsets within that class of genes can be achieved.

Once the component gene sequences are obtained, in this case the genes encoding the variable regions of the various heavy and light antibody chains, the light and heavy chain genes are associated in random combinations to form a random combinatorial library. Various recombinant DNA vector systems have been described to facilitate combinatorial cloning [see: PCT Publication No. WO90/14430 supra; Scott and Smith, Science 249:386-406 (1990); or U. S. Patent 5,223,409]. Having generated the combinatorial library, the products can, after expression, be conveniently screened by biopanning with RSV F protein or, if necessary, by epitope blocked biopanning as described in more detail below.

As described herein, it is preferred to use single chain antibodies for combinatorial cloning and screening and then to convert them to full length mAbs after selection of the desired candidate molecules. However, Fab fragments of mAbs can also be used for cloning and screening.

### *III. Antibody Fragments.*

The present invention contemplates the use of scFv, Fab, or F(ab')<sub>2</sub> fragments to derived full-length mAbs directed against the F protein of RSV. Although these

fragments may be independently useful as protective and therapeutic agents *in vivo* against RSV-mediated conditions or *in vitro* as part of an RSV diagnostic, they are employed herein as a component of a reshaped human antibody. A scFv fragment contains the light and heavy chain variable regions joined by a linker of about 12 amino acids in either a light-linker-heavy or a heavy-linker-light orientation. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain; and a F(ab')<sub>2</sub> fragment is the fragment formed by two Fab fragments bound by additional disulfide bonds. RSV binding monoclonal antibodies provide sources of scFv or Fab fragments which can be obtained from a combinatorial phage library [see, e.g., Winter et al., Ann. Rev. Immunol., 12:433-455 (1994) or Barbas et al., Proc. Nat'l. Acad. Sci. (USA) 89, 10164-10168 (1992), which are both hereby incorporated by reference in their entirety].

#### IV. Anti-RSV Antibody Amino Acid and Nucleotide Sequences of Interest.

The Fab G $\lambda$ -1 or other antibodies described herein may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

As one example, the present invention thus provides variable light chain and variable heavy chain sequences from the RSV human Fab G $\lambda$ -1A and sequences derived therefrom. The heavy chain variable region of Fab G $\lambda$ -1A is illustrated by Figs. 4, 8A-8F and 10A-10B [SEQ ID NOS: 3-4, 13 and 15].



The nucleic acid sequences of this invention, or fragments thereof, encoding the variable light chain and heavy chain peptide sequences are also useful for mutagenic introduction of specific changes within the nucleic acid sequences encoding the CDRs or framework regions, and for incorporation of the resulting modified or fusion nucleic acid sequence into a plasmid for expression. For example, silent substitutions in the nucleotide sequence of the framework and CDR-encoding regions can be used to create restriction enzyme sites which would facilitate insertion of mutagenized CDR (and/or framework) regions. These CDR-encoding regions may be used in the construction of reshaped human antibodies of this invention.

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences of the invention as well as functional fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, e.g., chimeric or humanized antibodies, or other engineered antibodies of this invention when operatively combined with a second immunoglobulin partner.

It should be noted that in addition to isolated nucleic acid sequences encoding portions of the altered antibody and antibodies described herein, other such nucleic acid sequences are encompassed by the present invention, such as those complementary to the native CDR-encoding sequences or complementary to the human framework regions surrounding the CDR-encoding regions. Such sequences include all nucleic acid sequences which

by virtue of the redundancy of the genetic code are capable of encoding the same amino acid sequence as given in Figs. 3 and 4 [SEQ ID NOS: 2 and 4]. Figs. 6 and 7 [SEQ ID NOS: 5-12] provide representations of such sequences. Other useful DNA sequences encompassed by this invention include those sequences which hybridize under stringent hybridization conditions [See: T. Maniatis et al., Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences encoding the G $\lambda$ -1 antibodies (e.g., sequences of Figs. 3, 4, 8A-8F through 11 [SEQ ID NOS: 1-4, 13-16]) and which retain the antigen binding properties of those antibodies. An example of one such stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for an hour. Alternatively an exemplary stringent hybridization condition is in 50% formamide, 4XSSC at 42°C. Preferably, these hybridizing DNA sequences are at least about 18 nucleotides in length, i.e., about the size of a CDR.

V. *Altered Immunoglobulin Coding Regions and Altered Antibodies.*

Altered immunoglobulin coding regions encode altered antibodies which include engineered antibodies such as chimeric antibodies, humanized, reshaped, and immunologically edited human antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions in the form of scFv regions that encode peptides having the antigen specificity of an RSV antibody, preferably a high affinity antibody such as provided by the present invention, inserted into an acceptor immunoglobulin partner.

When the acceptor is an immunoglobulin partner, as defined above, it includes a sequence encoding a second

antibody region of interest, for example, an Fc region. Immunoglobulin partners may also include sequences encoding another immunoglobulin to which the light or heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of RSV may be designed to elicit enhanced binding with the same antibody.

The immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the immunoglobulin partner may be operatively linked by conventional means.

Fusion or linkage between the immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art.

In addition, signal sequences for the molecules of the invention may be modified to enhance expression. For example the reshaped human antibody having the signal sequence and CDRs derived from the Fab G $\lambda$ -1 heavy chain sequence, may have the original signal peptide replaced with another signal sequence such as the Campath leader sequence [Page, M. J. et al., BioTechnology 9:64-68(1991)].

An exemplary altered antibody, a reshaped human antibody, contains a variable heavy and the entire light chain peptide or protein sequence having the antigen specificity of Fab G $\lambda$ -1, fused to the constant heavy regions C<sub>H-1</sub>-C<sub>H-3</sub> derived from a second human antibody.

In still a further embodiment, the engineered antibody of the invention may have attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the Fc fragment or C<sub>H-2</sub>C<sub>H-3</sub> domain of a complete antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any discrete fragment thereof, such as the Fab or F(ab')<sub>2</sub> fragments, a heavy chain dimer, or any minimal recombinant fragments thereof such as an F<sub>v</sub> or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor Fab G $\lambda$ -1. Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

Whenever the immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise immunoglobulin (Ig) constant regions and variable framework regions from one source, e.g., the acceptor antibody, and one or more (preferably all) CDRs from the donor antibody, e.g., the anti-RSV antibody described herein. In addition, alterations, e.g., deletions, substitutions, or additions, of the

acceptor mAb light and/or heavy variable domain framework region at the nucleic acid or amino acid levels, or the donor CDR regions may be made in order to retain donor antibody antigen binding specificity or to  
5 reduce potential immunogenicity.

Such engineered antibodies are designed to employ one (or both) of the variable heavy and/or light chains of the RSV mAb (optionally modified as described) or one or more of the below-identified heavy or light chain  
10 CDRs. The engineered antibodies of the invention are neutralizing, i.e., they desirably inhibit virus growth *in vitro* and *in vivo* in animal models of RSV infection.

Such engineered antibodies may include a reshaped human antibody containing the human heavy and light  
15 chain constant regions fused to the RSV antibody functional fragments. A suitable human (or other animal) acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology  
20 to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable  
25 framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not  
30 required to originate from the same acceptor antibody.

Desirably the heterologous framework and constant regions are selected from human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4), IgM, IgA and IgE. The Fc domains are not limited to native

sequences, but include mutant variants known in the art that alter function. For example, mutations have been described in the Fc domains of certain IgG antibodies that reduce Fc-mediated complement and Fc receptor binding [see, e.g., A. R. Duncan et al., Nature, 332:563-564 (1988); A. R. Duncan and G. Winter, Nature, 332:738-740 (1988); M.-L. Alegre et al., J. Immunol., 148:3461-3468 (1992); M.-H. Tao et al., J. Exp. Med., 178:661-667 (1993); and V. Xu et al. J. Biol. Chem., 269:3469-2374 (1994)]; alter clearance rate [J.-K. Kim et al., Eur. J. Immunol., 24:542-548 (1994)]; and reduce structural heterogeneity [S. Angal et al., Mol. Immunol. 30:105-108 (1993)]. Also, other modifications are possible such as oligomerization of the antibody by addition of the tailpiece segment of IgM and other mutations [R. I. F. Smith and S. L. Morrison, Biotechnology 12:683-688 (1994); R. I. F. Smith et al., J. Immunol., 154: 2226-2236 (1995)] or addition of the tailpiece segment of IgA [I. Kariv et al., J. Immunol., 157: 29-38 (1996)]. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

The altered antibody thus preferably has the structure of a natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use, e.g., treatment of RSV mediated diseases in man, or for diagnostic uses.

It will be understood by those skilled in the art that an altered antibody may be further modified by changes in variable domain amino acids without

necessarily affecting the specificity and high affinity of the donor antibody (i.e., an analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the  
5 variable domain frameworks or CDRs or both. Particularly preferred is the immunological editing of such reconstructed sequences as illustrated in the examples herein.

In addition, the variable or constant region may be  
10 altered to enhance or decrease selective properties of the molecules of the instant invention, as described above. For example, dimerization, binding to Fc receptors, or the ability to bind and activate complement [see, e.g., Angal et al., Mol. Immunol,  
15 30:105-108 (1993); Xu et al., J. Biol. Chem, 269:3469-3474 (1994); and Winter et al., EP 307,434-B].

Such antibodies are useful in the prevention and treatment of RSV mediated disorders, as discussed below.

#### 20 *VI. Production of Altered Antibodies and Engineered Antibodies.*

The resulting reshaped human antibodies of this invention can be expressed in recombinant host cells, e.g., COS, CHO or myeloma cells. A conventional expression vector or recombinant plasmid is produced by  
25 placing these coding sequences for the altered antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g.,  
30 CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is

identical to the first except insofar as the coding sequences and selectable markers are concerned. This ensures as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and  
5 light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to  
10 create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The production of the  
15 antibody which includes the association of both the recombinant heavy chain and light chain is measured in the culture by an appropriate assay, such as an enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA). Similar conventional techniques may be employed  
20 to construct other altered antibodies and molecules of this invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of  
25 skill in the art. For example, the conventional pUC series of cloning vectors, may be used. One vector used is pUC19, which is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Any vector,  
30 which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated, may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.



- Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vectors. Preferred vectors include for example plasmids pCD or pCN. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above described DNA sequences which code for the engineered antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.
- The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a polyadenylation (polyA) signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.
- The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or altered immunoglobulin molecules thereof. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

Suitable host cells or cell lines for the expression of the engineered antibody or altered antibody of the invention are preferably mammalian cells such as CHO, COS, a fibroblast cell (e.g., 3T3), and myeloid cells, and more preferably a CHO or a myeloid cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook *et al.*, Molecular Cloning (A Laboratory Manual), 2nd edit., Cold Spring Harbor Laboratory (1989).

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant scFvs, Fabs and MAb of the present invention [see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)]. The tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form does not pose as great a concern because Fabs are not normally glycosylated and can be engineered for exported expression, thereby reducing the high concentration that facilitates misfolding.

Nevertheless, any recombinant Fab produced in a bacterial cell would be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced and exported in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems [see, e.g. Miller et al., Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein].

The general methods by which the vectors of the invention may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the altered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the altered antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention.

Yet another method of expression of reshaped antibodies may utilize expression in a transgenic animal. An exemplary systems is described in U. S. Patent No. 4,873,316. The expression system described in that reference uses the animal's casein promoter and,

when transgenically incorporated into a mammal, permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the engineered antibody is then examined for *in vitro* activity by use of an appropriate assay. At present, conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the altered antibody to RSV. Additionally, other *in vitro* assays and *in vivo* animal models may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the altered antibody in the body despite the usual clearance mechanisms.

VII. Therapeutic/Prophylactic Uses.

This invention also relates to a method of treating humans experiencing RSV-related symptoms which comprises administering an effective dose of antibodies including one or more of the antibodies (altered, reshaped, monoclonal, etc.) described herein or fragments thereof.

The therapeutic response induced by the use of the molecules of this invention is produced by binding to RSV and thus subsequently blocking RSV propagation. Thus, the molecules of the present invention, when in preparations and formulations appropriate for therapeutic use, are highly desirable for those persons experiencing RSV infection. For example, longer treatments may be desirable when treating seasonal episodes or the like. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

The altered antibodies, antibodies and fragments thereof of this invention may also be used alone or in conjunction with other antibodies, particularly human or humanized mAbs reactive with other epitopes on the F protein or other RSV target antigens as prophylactic agents.

The mode of administration of the therapeutic and prophylactic agents of the invention may be any suitable route which delivers the agent to the host. The altered antibodies, antibodies, engineered antibodies, and fragments thereof, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly, intravenously, or intranasally.

Therapeutic and prophylactic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the altered antibody of the invention as an active ingredient in a pharmaceutically acceptable carrier. An aqueous suspension or solution containing the antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the engineered antibody of the invention or a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and

buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 80 mg, or more preferably, about 5 mg to about 75 mg, of an engineered antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 75 and preferably 5 to about 50 mg/ml of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the therapeutic and prophylactic agents of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat an inflammatory disorder in a human or other animal, one dose of approximately 0.1 mg to approximately 20 mg per 70 kg body weight of a protein or an antibody of this invention should be administered parenterally, preferably i.v. or i.m. (intramuscularly).

Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician.

The altered antibodies and engineered antibodies of this invention may also be used in diagnostic regimens, such as for the determination of RSV mediated disorders or tracking progress of treatment of such disorders. As diagnostic reagents, these altered antibodies may be conventionally labeled for use in ELISAs and other conventional assay formats for the measurement of RSV levels in serum, plasma or other appropriate tissue, or the release by human cells in culture. The nature of the assay in which the altered antibodies are used are conventional and do not limit this disclosure.

The antibodies, altered antibodies or fragments thereof described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

The following examples illustrate various aspects of this invention including the construction of exemplary engineered antibodies and expression thereof in suitable vectors and host cells, and are not to be construed as limiting the scope of this invention. All amino acids are identified by conventional three letter or single letter codes. All necessary restriction enzymes, plasmids, and other reagents and materials were obtained from commercial sources unless otherwise indicated. All general cloning ligation and other recombinant DNA methodology were as performed in T. Maniatis *et al.*, cited above, or Sambrook *et al.*, cited above.

**Example 1:** Isolation of G $\lambda$ -1 scFv-1

Single chain (sc) Fv libraries were prepared from an individual purposely exposed to RSV and selected against recombinant RSV F-protein following described procedures [R. H. Jackson *et al.*, in *Protein Engineering, A Practical Approach*, A. R. Rees *et al.* eds, Oxford University Press, chapter 12, pp. 277-301, 1992; H. R. Hoogenboom *et al.*, Nucl. Acid Res., 19: 4133-4137 (1991); J. D. Marks *et al.*, J. Mol. Biol., 222: 581-597 (1991)]. Briefly, lymphocytes were isolated from a blood sample taken 15 days post exposure. RNA isolated from the lymphocytes was used for preparation of scFv encoding repertoires for phage display. Sets of V-region primers were paired with constant region primers for heavy chain domain 1 IgG and IgM and light chain C-k and C- $\lambda$  and then linked in a scFv VH-VL orientation with a 15 amino acid spacer (glycine<sub>4</sub>-serine)<sub>3</sub> [SEQ ID NO: 21] by overlap PCR [see J. D. Marks *et al.*, cited above, for description of the primers].

The resulting four scFv repertoires (V-k with IgG and IgM, V- $\lambda$  with IgG and IgM) were cloned into a phagemid vector similar to pHEN1 [H. R. Hoogenboom *et al.*, cited above] resulting in fusion of the scFvs to gene III of phage fd. The vector was then transformed into *E. coli* (e.g., strain TG1) by electroporation to yield the corresponding phagemid libraries.

Phage libraries displaying the scFv-gene 3 fusions were prepared by infection of each of the plasmid libraries with the M13K07 helper phage [R. H. Jackson, cited above] and were individually subjected to 2 rounds of panning against recombinant F-protein coated onto plastic. In the first round, 10<sup>11</sup> phage in 2.5 ml phosphate buffered saline (PBS)/2% Marval™ non-fat dry



milk were incubated for 90 minutes in a tube coated with 5 µg/ml of F-protein [described in P. Tsui et al, J. Immunol., 157:772-780 (1996)] followed by 1 wash with 10x PBS/0.05% Tween 20 and a second wash with 10x PBS alone. Bound phage were eluted with 10 mM triethylamine and the eluate was neutralized with 1 M Tris-HCl, pH 7.4. The eluted phage were amplified and subjected to a similar second round of panning, except that the concentration of F-protein for coating was 2 µg/ml and the wash buffer contained 20x PBS.

*E. coli* were infected with the eluted phage and 96 colonies from each starting library were superinfected with helper phage and screened for F-protein binding activity. Only four positive clones were obtained from the 2 IgM libraries, whereas 41 positives were observed for the IgG libraries. By partial sequence analysis, all of the clones carried one of three different heavy chains. Complete sequences were obtained for the heavy and light chain V-regions for six clones, all from the IgG libraries.

Serial dilutions of titered phage stocks of each of these six clones were tested by ELISA for binding to recombinant F-protein and to RSV infected cell lysate. All showed binding to F-protein with the phage designated Gλ-1 showing the best activity. However, Gλ-1 and three other clones showed little binding to the RSV lysate.

Three clones: Gλ-1, Gλ-3 (lysate binding positive), and GK-1 (lysate binding negative), where "κ" and "λ" designate the class of the light chain, were characterized further for competition of their binding by F-protein specific neutralizing monoclonal antibodies, and their ability to inhibit virus

infection. The neutralizing mAbs RSV19 and B4 described in International patent publication No. WO92/04381, published March 19, 1992, and International patent publication No. WO93/20210, published October 14, 1993, recognize distinct epitopes on the F-protein. GK-1 was strongly inhibited by both antibodies. G $\lambda$ -1 was significantly inhibited by B4 only. GK-3 was not inhibited by either antibody (shown for G $\lambda$ -1 only; see Figs. 1A and 1B). In initial assays (Table I, experiments 1-3), all three clones showed neutralizing activity *in vitro*, with G $\lambda$ -1 being the most potent (Fig. 2, a graph of experiment 2), while control wild-type phage (M13K07) not displaying scFv had no effect.

To address the possibility that neutralization might result just from phage coating of virus, irrespective of epitope, a phage preparation of the non-neutralizing Fab 5-16 was tested in the same assay. In three out of four assays, this preparation also showed good neutralization activity, as did the control phage in two of these assays (Table I, experiments 4-7). This confounding observation of variable neutralization by both Fab 5-16 and control M13K07 phage rendered the viral neutralization studies inconclusive.

Table I

Phage Sample	Virus Neutralization ( $IC_{50} \times 10^{-3}$ ) <sup>1</sup> (aru or kru/ml) <sup>2</sup>						
	Experiment #						
	1	2	3	4	5	6	7
Gk-1 a	1,600		<300				
b				<10	<7		
Gλ-1 a		80	<300				
b				8.1	11		
c							120
Gλ-3 a		900	<300	180			
b					<7	10	
c							730
M13K07a			>10 <sup>b</sup>	>10 <sup>b</sup>		>5,000	
b					+all dil.	+all dil.	>10 <sup>c</sup>
Fab 5-19a				>10 <sup>b</sup>	40	180	
b							3.5

## Legend:

<sup>1</sup> Assay according to M. J. Cannon, J. Virol. Meth., 16:293-301. Virus at 100 infectious centers/well was incubated with dilutions of the indicated phage for 1 hr and then added to susceptible cells for 3 hr. The virus/phage solution was aspirated and replaced with fresh medium and the cells were incubated overnight before peroxidase staining for virus infected cells.

<sup>2</sup> aru = ampicillin resistance units, a measure of phagmid containing particles.

kru = kanamycin resistance units, a measure of particles containing the phage genome (for the M13K07 control only).

In the face of these results, made more ambiguous by the dependence of all assays on phage stocks versus antibody proteins of known concentration, G $\lambda$ -1 was selected as the most likely candidate for a potent neutralizing antibody based on (1) its apparent better binding to F-protein, (2) its selective inhibition of binding by the B4 antibody, and (3) its suggested activity over background in the virus neutralization assay.

**Example 2: Conversion of G $\lambda$ -1 scFV to mAb Version A**

The DNA and encoded protein sequences of the VH and VL regions of G $\lambda$ -1 are shown in Figs. 3 [SEQ ID NOS: 1 and 2] and 4 [SEQ ID NOS: 3 and 4], respectively. For expression in mammalian cells, the heavy chain variable region and the light chain variable region from the G $\lambda$ -1 plasmid were cloned into derivatives of plasmid pCDN [Nambi, A. et al., Mol. Cell. Biochem., 131:75-86 (1994)] in which the expression of the antibody chain is driven by the cytomegalovirus promoter (CMV) promoter. Plasmid pCD-HC68B is used for expressing full length heavy chains and plasmid pCN-HuLC, for expressing full length light chains.

In the initial constructs, changes in the sequence at the amino terminus were introduced by the PCR primers used for cloning the light chain and heavy chain variable regions from plasmid G $\lambda$ -1. In these constructs, the peptide signal sequence for both the heavy and light chains is derived from the Campath light chain [M. J. Page et al., Biotechnology 9: 64-68 (1991)]. The heavy chain of G $\lambda$ -1 was PCR amplified from G $\lambda$ -1 phagemid DNA, using primers for the amino terminus and framework 4 of the variable region. The resulting

PCR fragment was cut with XhoI (site introduced by the amino terminus primer) and BstEII (naturally occurring site in framework 4), and cloned into an intermediate vector, F4HCV, at the XhoI/BstEII sites.

5           This cloning grafted the variable region of G $\lambda$ -1 onto the constant region of another anti-RSV heavy chain 194-F4 [cloned at SmithKline Beecham from a human hybridoma]. This intermediate clone was cut with XhoI and Bsp120I, and introduced into the same sites in pCD-  
10 HC68B. The XhoI site is introduced at the amino terminus by the PCR primer and, when cloned into pCD-HC68B at the same site is preceded in frame by the Campath leader sequence. The Bsp120I site is a naturally occurring, highly conserved sequence at the  
15 beginning of the C<sub>H-1</sub> domain, and when cloned into pCD-HC68B at the same site, is in frame with the remaining sequence for the C<sub>H-1</sub> through C<sub>H-3</sub> regions of human IgG<sub>1</sub>. In the resulting construct, G $\lambda$ -1Ap<sub>CD</sub> (Figs. 8A-8F [SEQ ID NO: 13]), the amino acids immediately following the  
20 Campath leader are EVQLLE [SEQ ID NO: 17], where the residues LE are encoded by the nucleotide sequence for the XhoI cloning site.

          The light chain of G $\lambda$ -1 was PCR amplified from the G $\lambda$ -1 phagemid DNA, using primers for the amino terminus  
25 and framework 4 of the variable region. The resulting PCR fragment was cut with SacI (site introduced by the amino terminus primer) and AvrII (naturally occurring site in framework 4), and cloned into 43-lpcn at the SacI/AvrII sites. This cloning grafted the variable  
30 region of G $\lambda$ -1, in frame, onto the constant region of another anti-RSV lambda light chain 43 [P. Tsui et al., J. Immunol., 157: 772-780 (1996)], which had been cloned at SmithKline Beecham from a combinatorial library derived from RNA isolated from human spleen. The SacI

site is introduced at the amino terminus by the PCR primer and, when cloned into 43pcn at the same site, is preceded in frame by the Campath leader sequence. The first two amino acids of the mature light chain are therefore deleted. In the resulting construct, G $\lambda$ -1Apcn (Figs. 9A-9E [SEQ ID NO: 14]), the first two amino acids immediately following the leader are EL, where the residues EL are encoded by the nucleotide sequence for the SacI cloning site.

The nucleotide sequences of the plasmids G $\lambda$ -1Apcd and G $\lambda$ -1Apcn are shown in Figs. 8A-8F [SEQ ID NO: 13] and 9A-9E [SEQ ID NO: 14] respectively. This set of vectors was used to produce antibody G $\lambda$ -1A in COS cells and in CHO cells.

15

**Example 3: Cloning Of The Corrected G $\lambda$ -1 Heavy and Light Chains**

In cloning the variable region of the G $\lambda$ -1 heavy chain from the single chain Fv (scFv) format into the full length format, the fifth amino acid at the amino terminus was changed from Val to Leu, for cloning purposes. To correct this change, PCR primers were designed for the amino terminus of the G $\lambda$ -1 heavy chain cloned into pCD, which reverted the fifth amino acid back to Val. The correction was introduced via the PCR overlap technique using the correction primers and primers annealing to sequences within the CMV promoter and the C<sub>H-2</sub> constant region as the outside 5' and 3' primers, respectfully. The final PCR product was digested with restriction enzymes, EcoRI and Bsp120I, and cloned into the G $\lambda$ -1Apcd vector at the same sites to create G $\lambda$ -1Bpcd.

The final construct was sequenced to verify that the amino terminus of the heavy chain had been corrected from EVQLLE [SEQ ID NO: 17] to EVQLVE [SEQ ID NO: 18] (see Fig 6). The nucleotide sequence of coding region  
5 for the corrected heavy chain, G $\lambda$ -1B, is shown in Figs. 10A-10B [SEQ ID NO: 15].

In cloning the variable region of the G $\lambda$ -1 light chain from the scFv format into the full length format, changes were introduced at the amino terminus for  
10 cloning purposes. Specifically, the first 2 amino acids (Gln and Ser) of the light chain were deleted and the third amino acid was changed from Val to Glu. To correct these changes, PCR primers were designed for the amino terminus of the G $\lambda$ -1 light chain cloned into pCn,  
15 which replaced the two deleted amino acids (Gln and Ser) and reverted the third amino acid back to Val. The corrections were introduced via the PCR overlap technique using the correction primers and primers annealing to sequences within the CMV promoter and the  $\lambda$   
20 constant region as the outside 5' and 3' primers, respectfully. The final PCR product was digested with restriction enzymes, EcoRI and AvrII and cloned into the G $\lambda$ -1ApCn vector at the same sites to create G $\lambda$ -1BpCn.

The final construct was sequenced to verify that  
25 the amino terminus of the light chain had been corrected from --EL to QSVL (amino acids 1-4 of SEQ ID NO: 10).

The nucleotide sequence of coding region for the corrected light chain, G $\lambda$ -1B, is shown in Fig. 11 [SEQ ID NO: 16]. This vector G $\lambda$ -1BpCn, was used with G $\lambda$ -  
30 1BpC to produce antibody G $\lambda$ -1B, in COS cells and in CHO cells.

**Example 4: Production of G $\lambda$ -1 mAbs in Mammalian Cells**

For initial characterization, the mAb constructs for each version, G $\lambda$ -1A heavy and light chain, G $\lambda$ -1B heavy and light chain, were expressed in COS cells essentially as described in Current Protocols in Molecular Biology, eds F. M. Ausubel et al., 1988, John Wiley & Sons, vol. 1, section 9.1. On day 1 after the transfection, the culture growth medium was replaced with a serum-free medium [SmithKline Beecham] which was changed on day 3. Similar satisfactory results are obtained using a publicly available medium, DMEM supplemented with ITS<sup>TM</sup> Premix, an insulin, transferrin, selenium mixture (Collaborative Research, Bedford, MA) and 1 mg/ml bovine serum albumin (BSA).

The mAb was prepared from the day 3 + day 5 conditioned medium by standard protein A affinity chromatography methods (e.g., as described in Protocols in Molecular Biology) using, for example, Prosep A affinity resin (Bioprocessing Ltd., UK).

To produce larger quantities of the G $\lambda$ -1B mAb (100-200 mgs), the vectors were introduced into a proprietary CHO cell system. However, similar results will be obtained using dhfr<sup>-</sup> CHO cells as previously described [P. Hensley et al., J. Biol. Chem., 269:23949-23958 (1994)]. Briefly, a total of 30  $\mu$ g of linearized plasmid DNA (15  $\mu$ g each of the A or B set of heavy chain and light chain vectors) is electroporated into  $1 \times 10^7$  cells. The cells are initially selected in nucleoside-free medium in 96 well plates. After three to four weeks, media from growth positive wells is screened for human immunoglobulin using an ELISA assay. The highest expressing colonies are expanded and selected in increasing concentrations of methotrexate for



amplification of the transfected vectors. The antibody is purified from conditioned medium by standard procedures using protein A affinity chromatography (Protein A sepharose, Pharmacia) followed by size  
5 exclusion chromatography (Superdex 200, Pharmacia).

The concentration and the antigen binding activity of the eluted antibody are measured by ELISA. The antibody containing fractions are pooled and further purified by size exclusion chromatography. As expected  
10 for any such antibody, by SDS-PAGE, the predominant protein product migrated at approximately 150 kd under non-reducing conditions and as two bands of 50 and 25 kd under reducing conditions. For antibody produced in CHO cells, the purity was > 90%, as judged by SDS-PAGE, and  
15 the concentration was accurately determined by amino acid analysis.

**Example 5: Binding of the G $\lambda$ -1 mABs to recombinant F protein**

20 Binding of the G $\lambda$ -1 mABs to recombinant F protein was measured in a standard solid phase ELISA. Antigen diluted in PBS pH 7.0 was adsorbed onto polystyrene round-bottom microplates (Dynatech, Immunolon II) for 18 hours. Wells were then aspirated and blocked with 0.5%  
25 boiled casein (BC) in PBS containing 1% Tween 20 (PBS/0.05% BC) for two hours. Antibodies (50  $\mu$ l/well) were diluted to varying concentrations in PBS/0.5% BC containing 0.025% Tween 20 and incubated in antigen coated wells for one hour. Plates were washed three  
30 times with PBS containing 0.05% Tween 20, using a Titertek 320 microplate washer, followed by addition of HRP-labelled protein A/G (50  $\mu$ l) diluted 1:5000. After washing three times, TMBBlue substrate (TSI, #TM102) was added and plates were incubated an additional 15

minutes. The reaction was stopped by addition of 1 N H<sub>2</sub>SO<sub>4</sub> and absorbance read at 450 nm using a Biotek ELISA reader.

The antigen binding epitope of the Gλ-1 mAbs was  
5 examined in a competition ELISA. The Gλ-1 mAbs were mixed with increasing concentrations of RSMU19 or B4, two potent neutralizing mAbs [Tempest *et al.*, Biotech., 9: 266-271 (1991); Kennedy *et al.*, J. Gen. Virol., 69: 3023-3032 (1988)] and added to F protein-coated wells.  
10 The epitope regions recognized by mAbs RSMU19 and B4 are quite distinct from each other as previously described in Arbiza *et al.*, J. Gen. Virol., 73: 2225-2234 (1992). The concentration of the Gλ-1 mAbs used in competition studies was determined previously to give 90% maximal  
15 binding to F antigen. Binding of the Gλ-1 mAbs in the presence of other mAbs was detected using HRP-labelled goat anti-human IgG. The reaction was developed as stated above.

The Gλ-1 mAbs demonstrated potent binding to  
20 recombinant F (rF) protein by ELISA (EC<sub>50</sub> for mAb B = 2.6 ng/ml). Binding of the Gλ-1 mAbs to rF protein was inhibited by mAb B4, for which the F protein amino acids critical for antigen recognition are amino acids 268, 272 and 275 of SEQ ID NO: 20). Binding of the Gλ-1 mAbs  
25 to rF protein was not inhibited by mAb RSMU19, for which F protein amino acid 429 of SEQ ID NO: 20 is critical for antigen recognition. These results indicate that residues in the region of amino acids 255-275 of the F protein [SEQ ID NO: 20] are critical for Gλ-1 mAb  
30 recognition.

**Example 6:** In vitro Fusion-Inhibition Activity of the  
Gλ-1 mABs

The ability of the Gλ-1 mABs to inhibit virus-induced cell fusion was determined using a modification of the *in vitro* microneutralization assay [Beeler et al., *J. Virol.*, 63:2941-2950 (1989)]. In this assay, 50 μl of RS Long strain virus (10-100 TCID<sub>50</sub>/well [American Type Culture Collection ATCC VR-26] were mixed with 0.1 ml VERO cells (5X10<sup>3</sup>/well) [ATCC CCL-81] in Minimum Essential Media (MEM) containing 2% fetal calf serum (FCS), for 4 hours at 37°C, 5% CO<sub>2</sub>. Serial two-fold dilutions (in quadruplicate) of mAB (50 μl) were then added to wells containing virus-infected cells. Control cultures contained cells incubated with virus only (positive virus control) or cells incubated with media alone.

Cultures were incubated at 37°C in 5% CO<sub>2</sub> for 6 days at which time cytopathic effects (CPE) in virus control wells were > 90%. Microscopic examination for cytopathic effects were confirmed by ELISA. Media was aspirated from cultures and replaced with 50 μl of 90% methanol containing 0.6% H<sub>2</sub>O<sub>2</sub>. After 10 minutes, fixative was aspirated and plates were air dried overnight. Viral antigen was detected in the fixed cultures using 1 μg/ml biotinylated RSCB4 (a human Fc derivative of the bovine B4 mAb [SmithKline Beecham]), followed by HRP-labelled streptavidin (Boehringer-Mannheim) diluted 1:10,000. The reaction was developed using TMBlue and stopped by addition of 1N H<sub>2</sub>SO<sub>4</sub>. Absorbance was measured at 450 nm (O.D.<sub>450</sub>).

Fusion-inhibition titers were defined as the concentration of antibody which caused a 50% reduction in ELISA signal (ED<sub>50</sub>) as compared to virus controls.

Based on the curve generated in the ELISA by the standard virus titration, a 50% reduction in O.D.<sub>450</sub> corresponded to  $\geq$  90% reduction in virus titer. Calculation of the 50% point was based on regression analysis of the dose titration.

The G $\lambda$ -1 mABs demonstrated potent *in vitro* fusion-inhibition activity against type A RS Long strain virus (ED<sub>50</sub> for mAB B of 0.51  $\pm$  0.38  $\mu$ g/ml). In this *in vitro* fusion-inhibition assay, G $\lambda$ -1 mAB B was more active than the humanized mAB RSHZ19 (ED<sub>50</sub> of 0.4-3.0  $\mu$ g/ml) [Wyde et al., Pediatr. Res., 38(4):543-550] in comparative assays.

**Example 7: In vivo Activity of G $\lambda$ -1 mAB B: Prophylaxis and Therapy in Balb/c Mouse Model**

Balb/c mice (5/group) were inoculated intraperitoneally with doses ranging from 0.06 mg/kg to 5 mg/kg of G $\lambda$ -1 mAB B either 24 hours prior (prophylaxis) or 4 days after (therapy) intranasal infection with 10<sup>5</sup> PFU of the A2 strain of human RSV. Mice were sacrificed 5 days after infection. Lungs were harvested and homogenized to determine virus titers.

Virus was undetectable in the lungs of mice treated prophylactically with  $\geq$  1.25 mg/kg G $\lambda$ -1 mAB B either prophylactically or therapeutically. See Table II below. Significant viral clearance (2-3 log<sub>10</sub>) was also achieved in animals receiving 0.31 mg/kg G $\lambda$ -1 mAB B either prophylactically or therapeutically.

**Table II: G $\lambda$ -1 mAB B Prophylaxis and Therapy in Balb/c Mice**

	<u>Treatment</u>	<u>Dose</u> (mg/kg)	<u>Lung Virus Titer (log<sub>10</sub>/g lung)</u>	
			<u>Prophylaxis</u>	<u>Therapy</u>
5	<b>G<math>\lambda</math>-1 mAB B</b>	5	<1.7	<1.7
		1.25	<1.7	<1.7
		0.31	1.8 $\pm$ 0.3	2.9 $\pm$ 0.4
		0.06	4.3 $\pm$ 0.7	4.5 $\pm$ 0.3
10	<b>PBS</b>	-	4.8 $\pm$ 0.7	4.7 $\pm$ 0.2

The G $\lambda$ -1 mABs have potent antiviral activity *in vitro* against a broad range of native RSV isolates of both type A and B, and show prophylactic and therapeutic efficacy *in vivo* in animal models. Thus, the G $\lambda$ -1 mABs are candidates for therapeutic, prophylactic, and diagnostic application in man.

Numerous modifications and variations of the present invention may be made by one of skill in the art in view of the invention described herein. Such modifications are believed to be encompassed by the specification and claims of the present invention. All references cited above are incorporated by reference herein.

## WHAT IS CLAIMED IS:

1. A human monoclonal antibody and functional fragments thereof, specifically reactive with an F protein epitope of Respiratory Syncytial Virus and capable of neutralizing infection by said virus selected from the group consisting of G $\lambda$ -1A and G $\lambda$ -1B.

2. The monoclonal antibody according to Claim 1 which comprises the light chain amino acid sequence of Fig. 3 SEQ ID NO: 2 and the heavy chain amino acid sequence of Fig. 4 SEQ ID NO: 4.

3. The monoclonal antibody according to Claim 1 which comprises the light chain amino acid sequence encoded by the DNA sequence of Fig. 11 SEQ ID NO: 16 and the heavy chain amino acid sequence encoded by the DNA sequence of Figs. 10A-10B SEQ ID NO: 15.

4. The monoclonal antibody according to Claim 1 wherein said fragment is selected from the group consisting of Fv, Fab and F(ab')<sub>2</sub>.

5. An isolated nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid sequence encoding any of the human monoclonal antibodies, altered antibodies and CDRs of any of the claims 1-4;

(b) a nucleic acid complementary to any of the sequences in (a); and

(c) a nucleic acid sequence of 18 or more nucleotides capable of hybridizing to the CDRs of any of claims 1-4 under stringent conditions.

6. The isolated nucleic acid molecule according to Claim 5 comprising the sequences of Figs. 8A-8F and 9A-9E SEQ ID NOS: 13 and 14, or Figs. 10A-10B and 11 SEQ ID NOS: 15 and 16.

7. A recombinant plasmid comprising the nucleic acid sequences of any of Claims 5 or 6.

8. A host cell comprising the plasmid of Claim 7.

9. A process for the production of a human antibody specific for RSV comprising culturing the host cell of Claim 8 in a medium under suitable conditions of time temperature and pH and recovering the antibody so produced.

10. A method of detecting RSV comprising contacting a source suspected of containing RSV with a diagnostically effective amount of the monoclonal antibody of Claim 1 and determining whether the monoclonal antibody binds to the source.

11. A method for providing passive immunotherapy to RSV disease in a human, comprising administering to the human an immunotherapeutically effective amount of the monoclonal antibody of Claim 1.

12. The method according to Claim 11 wherein the passive immunotherapy is provided prophylactically.

13. A pharmaceutical composition comprising at least one dose of an immunotherapeutically effective

amount of the monoclonal antibody of Claim 1 in a pharmaceutically acceptable carrier.

14. A pharmaceutical composition comprising at least one dose of an immunotherapeutically effective amount of the monoclonal antibody of Claim 1 in combination with at least one additional monoclonal antibody.

15. The pharmaceutical composition according to Claim 14 wherein said additional monoclonal antibody is an anti-RSV antibody distinguished from the antibody of Claim 1 by virtue of being reactive with a different epitope of the RSV F protein antigen.



Fig. 1A

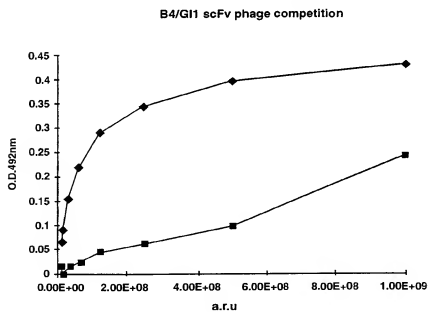
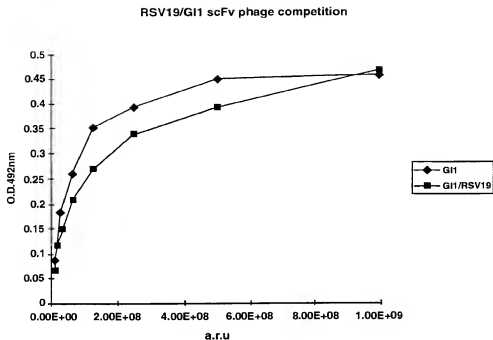


Fig. 1B

Fig. 2

## Neutralisation of RS/V/273 with phage Fv

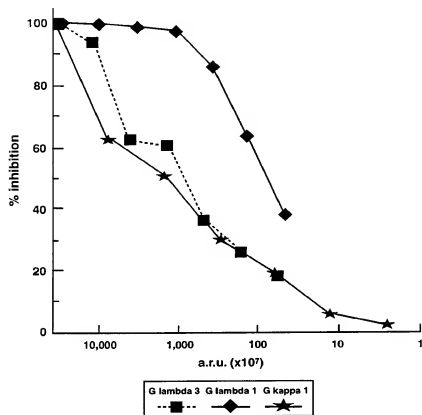


FIGURE 3

1	CAGTCTGTGTTGACGCAGCCGCCCTCAGTCTCTGCGGCCCCAGGACAGAA	50
	Q S V L T Q P P S V S A A P G Q K	
51	GGTCACCATCTCCTGCACTGGGAGCAGCTCCAACCTCGGGGCAGGTATG	100
	V T I S C T G S S S N L G A G Y D	
101	ATGTTCACTGGTACCGGCAACTTCCAGGGACAGCCCCCAAACCTCCTCATC	150
	V H W Y R Q L P G T A P K L L I	
151	TATGATAACAACAATCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTC	200
	Y D N N N R P S G V P D R F S G S	
201	CAAGTCTGGCCCCCTCAGCCTCCCTGGCCATCTCTGGGCTCCAGGCTGAGG	250
	K S G P S A S L A I S G L Q A E D	
251	ATGAGGCTGATTATTACTGCCAGTCCTATGACAGCAGCCTGAATGGTTAT	300
	E A D Y Y C Q S Y D S S L N G Y	
301	GTCTTCGGAACCTGGGACCCAGCTCACCGTCCTAGGT	336
	V F G T G T Q L T V L G	

FIGURE 4

1	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTC	50
	E V Q L V E S G G G L V Q P G G S	
51	CCTGAGACTCTCCTGCGCAGCCTCTGGAGTCTCCCTCAGTGGATACAAGA	100
	L R L S C A A S G V S L S G Y K M	
101	TGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGGTCTCTTCC	150
	N W V R Q A P G K G L E W V S S	
151	ATTACTGGTATGAGTAATTACATACACTACTCAGACTCAGTGAAGGGCCG	200
	I T G M S N Y I H Y S D S V K G R	
201	ATTCAACATCTCCAGAGACAACGCCATGAACTCACTGTATCTGCAAATGA	250
	F T I S R D N A M N S L Y L Q M N	
251	ACAGCCTGACAGCCGAGGACACGGGTGTTTATTATTGTGCGACACAACCG	300
	S L T A E D T G V Y Y C A T Q P	
301	GGGGAGCTGGCGCCTTTTGACCATTTGGGGCCAGGGAACCCCTGGTCACCGT	350
	G E L A P F D H W G Q G T L V T V	
351	CTCCTCA	357
	S S	

Figure 5

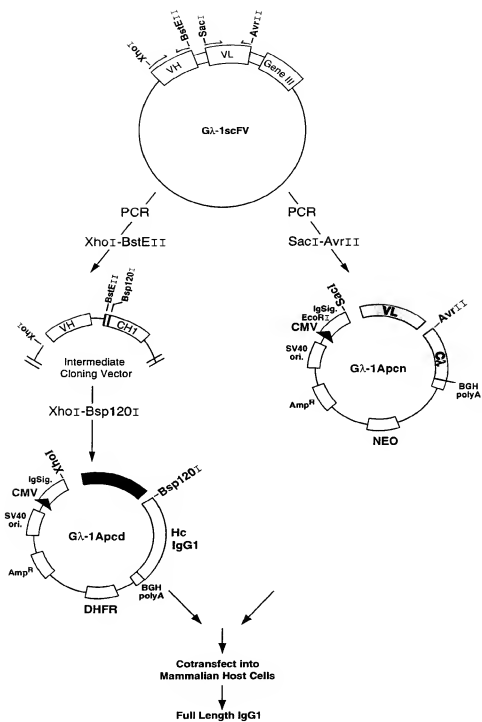


FIGURE 6

**Comparison of the Heavy Chain Amino Acid Sequences of the  
Gλ-1 single chain fv and mAbs**

Leader and Variable Regions

```

GL Dp58:                      EVQLVESGGGLVQPGGSLRLSCAASGFTFS
Gλ-1 scFv:                    -----VSL-
Gλ-1A:      MGWSCIILFLVATATGVHS---L-----
Gλ-1B:      -----V-----

                                CDR1                CDR2
                                -----
GL Dp58:      SYEMNWVRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLY
Gλ-1 scFv:    G-K-----S-TGMSNY-H-S-----M---
Gλ-1A:      -----
Gλ-1B:      -----

                                CDR3
                                -----
GL: Dp58:      LQMNSLRAEDTAVYYCAR
Gλ-1 scFv:    -----T-----G-----TQPGELAPFDHWGQGLVTVSS
Gλ-1A:      -----
Gλ-1B:      -----

```

FIG 7

**Comparison of the Light Chain Amino Acid Sequences of the G $\lambda$ -1A:  
single chain Fv and mAbs**

Leader and Variable Regions

		CDR1
		-----
GL DpL8:	QSVLTQPPSVSGAPGQRVTISC	<b>TGSSSNIG</b>
G $\lambda$ -1 scFv:	-----A----	K-----L-
G $\lambda$ -1A:	<b>MGWSCIIILFLVATATGVHS</b>	E-----
G $\lambda$ -1B:	-----QSV-----	
		CDR2
		-----
GL DpL8:	<b>AGYDVH</b> WYQQLPGTAPKLLIY <b>QNSNRFS</b> GV	PDRFSGSKSGTSASLAITGL
G $\lambda$ -1 scFv:	-----R-----D-N-----	P-----S--
G $\lambda$ -1A:	-----	
G $\lambda$ -1B:	-----	
		CDR3
		-----
GL DpL8:	QAEDADYYC	
G $\lambda$ -1 scFv:	-----QSYDSSLNGYV	FGTGTQLTVLG
G $\lambda$ -1A:	-----	
G $\lambda$ -1B:	-----	

FIGURE 8A

```

1   gacgctcgcgccgctctagggcctccaaaaaagcctcctcactacttctg
51  aatagctcagaggccgagggcgccctcgccctctgcataataaaaaaat
101 tagtcagccatgcatggggcgagaaatgggcggaactggcgaggttagg
151 ggcgggatggggcgaggttagggcgggactatgggttctgactaattgag
201 atgcatgctttgcatacttctgcctgctggggagcctggggactttccac
251 acctgggttctgactaattgagatgcatgctttgcatacttctgcctgct
301 ggggagcctggggactttccacaccctaactgacacacattccacagaat
351 taattcccggggatcgatccgctcgacgtacgactagttattaatagtaat
401 caattacggggtcattagttcatagcccataatatggagttccgcgttaca
451 taacttacggtaaatggcccgctggctgaccgccaacgacccccgcc
501 attgacgtcaataatgacgtatgttcccatagtaacgccaatagggactt
551 tccattgacgtcaatgggtggactatttacggtaaactgccacttggca
601 gtacatcaagtgtatcatatgccaaagtacgccccctattgacgtcaatga
651 cggtaaatggcccgctggcattatgcccgatcatgaccttatgggact
701 ttcttacttggcagttacatctacgtattagtcacgctattaccatgggtg
751 atgcgggttttggcagttacatcaatgggcgtggatagcgggtttgactcacg
801 gggattttccaagtctccacccattgacgtcaatgggagtttgttttggc
851 accaaaatcaacgggactttccaaaatgtcgtacaacactccgccccattg
901 acgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagc
                                     EcoRI
951 tgggtacgtgaaccgtcagatcgccctggagacgccatcgaattctgagca
1001 cacaggacctcaccatgggatggagctgtatcatcctcttcttggtagca
      M G W S C I I L F L V A
      Leader start

                                     XhoI
1051 acagctacagggtgtccactccgaggtccaactgctcgagttctgggggagg
      T A T G V H S E V Q L L E S ---
      Processed N-term

```



FIGURE 8B

1101 cttgggtacagcctgggggggtccctgagactctctctgcgagcctctggag  
1151 tctccctcagtggtatataagatgaactgggtccgccagggtccaggaag  
1201 ggggtggaatgggtctcttccattactggtatgagtaattacatacacta  
1251 ctcagactcagtggaagggccgattcaccatctccagagacaacgccatga  
1301 actcactgtatctgcaaataaacagcctgacagccgaggacacgggtgtt  
1351 tattattgtgcgacacaaccggggggagctggcgcccttttgaccattgggg  
1401 ccaggaaccctgggtcaccgtctctcagcctccaccaaggggcccatcgg  
Q G T L V T V S S /  
framework IV / CH1  
1451 tcttccccctggcaccctctctcaagagcacctctggggggcacagcgcc  
1501 ctgggctgctgggtcaaggactacttccccgaaccgggtgacgggtgctgtg  
1551 gaactcaggcgccctgaccagcggtgacacacctcccggtgtctctac  
1601 agtctcaggaactctactccctcagcagcgtgggtgaccgtgcctccagc  
1651 agcttgggcacccagacctacatctgcaacgtgaatcacaaagccagcaa  
1701 caccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcaca  
1751 catgcccaccgtgcccagcacctgaactcctggggggacgctcagttctt  
1801 ctcttccccccaaaaccaaggacacctcatgatctcccgacccctga  
1851 ggtcatatgcgtgggtggagctgagccacgaagacctgaggtcaagt  
1901 tcaactggtagctggacggcggtgaggtgcataatgccaaagacaaagccg  
1951 cgggaggagcagtagaacagcacgtaccgggtggtagcgtcctcacgct  
2001 cctgcaccaggactgggtgaatggcaaggagtacaagtgaaggctctcca  
2051 acaaaagccctcccagcccccatcgagaaaaccatctccaaagcgaaggg  
2101 cagccccgagaaccacaggtgtacacctgcccccatccgggatgagct  
2151 gaccaagaaccaggtcagcctgacctgctgggtcaaaggcttctatccca

FIGURE 8C

2201 ggcacatcgccgtggagtgggagagcaatgggcagccggagaacaactac  
2251 aagaccacgcctcccgtgctggactccgacggctccttcttctctacag  
2301 caagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcat  
2351 gctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc  
2401 tcctgtctccgggtaaatgtagatatactacgtatgatcagcctcgact  
S P G K \* C-term of heavy chain  
2451 gtgccttctagttgccagccatctgttgtttgccctcccccgtccttc  
2501 cttgaccctggaagggtgccactcccactgtcctttcttaataaaatgagg  
2551 aaattgcatcgcatgtctgagtaggtgtcattctattctggggggtggg  
2601 gtggggcaggacagcaagggggaggattgggaagacaatagcaggcatgc  
2651 tggggatgcggtgggctctatggaaccagctggggctcgacagcgctgga  
2701 tctccgatccccagctttgctctctcaatttctatttgcataatgagaa  
2751 aaaaaggaaaattaattttaacaccaattcagtagttgattgagcaaatg  
2801 cgttgccaaaaaggatgcttttagagacagtgtctctgcacagataagga  
2851 caaacattattcagagggagtaccagagctgagactcctaagccagtga  
2901 gtggcacagcattctagggagaaatatgcttgtcatcccaagcctgat  
2951 tccgtagagccacaccttggttaagggccaatctgctcacacaggatagag  
3001 agggcaggagccagggcagagcatataaggtaggtaggatcagttgctc  
3051 ctcacatttgcttctgacatagttgtgtgggagcttgatagcttggac  
3101 agctcagggtgctgcatcttcgcgccaacttgacggcaatcctagcgtgaa  
3151 ggcgtgtaggattttatccccgctgccatcatggttcgaccattgaactg  
3201 catcgctgccgtgtccaaaatatggggattggcaagaacggagacctac  
3251 cctggcctccgctcaggaaacgagttcaagtacttccaaagaatgaccaca  
3301 acctcttcagtggaaggtaaacagaatctggtgattatgggtaggaaaaac  
3351 ctggttctccattcctgagaagaatcgacctttaaggacagaattaata

FIGURE 8D

3401 tagttctcagtagagaactcaagaaccaccacgaggagctcattttctt  
3451 gccaaaagtttggatgatgccttaagacttattgaacaaccggaattggc  
3501 aagtaaagtagacatgggttgatagtcggaggcagttctgtttaccagg  
3551 aagccatgaatcaaccaggccaccttagactctttgtgacaaggatcatg  
3601 caggaaatttgaaagtgcacggtttttcccagaaattgatttggggaaata  
3651 taaacttctcccagaataaccaggcgctctctctgagggtccaggaggaaa  
3701 aaggcatcaagtataagtttgaagtctacgagaagaaagactaacaggaa  
3751 gatgctttcaagttctctgctccccctcctaaagctatgcatttttataag  
3801 accatgggacttttgctggctttagatcagcctcgactgtgccttctagt  
3851 tgccagccatctgttgttttggccctcccccgctcctcttgaccctgga  
3901 aggtgccactcccactgtcctttcctaataaaaatgaggaaattgcatcgc  
3951 attgtctgagtaggtgtcattctattcttgggggtgggggtggggcaggac  
4001 agcaagggggaggattgggaagacaatagcaggcatgtcggggatgcgggt  
4051 gggctctatggaaccagctggggctcgatcgatgtatgactgcggccgc  
4101 gatcccgctcgagagcttggcgtaatcatggtcatagctgtttcctgtgtg  
4151 aaattgttatccgctcacaaattccacacaacatacgagccggaagcataa  
4201 agtgtaaagcctgggggtgcctaatagtagtgaactaacattaatggc  
4251 ttgcgctcactgccccgtttccagtcgggaaacctgtcgtgccagctgca  
4301 ttaatgaatcggccaacgcgcggggagagggcgtttgcgtattgggcgct  
4351 ctccgcgttctcgtcactgactcgctgcgctcggtcgttcgggtcggg  
4401 cgagcgggtatcagctcactcaaaggcggtaatacgggttatccacagaatc  
4451 aggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggcca  
4501 ggaaccgtaaaaaggccgcgttgctggcggtttttccataggctccgcccc  
4551 cctgacgagcatcacaaaaatcgacgctcaagtcagagggtggcgaaacct  
4601 gacaggactataaagataccaggcggtttccccctggaagctccctcgtgc

FIGURE 8E

4651 gctctcctgttccgacccctgccgcttacccgataacctgtccgcctttctc  
4701 ccttcgggaagcgtggcgctttctcaatgctcacgctgtaggtatctcag  
4751 ttcggtgttaggtcgttcgctccaagctgggctgtgtgcacgaacccccg  
4801 ttcagcccgaccgctgcgccttatccggtaacctatcgctcttgagttccaac  
4851 ccggttaagacacgacttatcgccactggcagcagccactggtaacaggat  
4901 tagcagagcgcgaggtatgtaggcgggtgtacagagttcttgaaagtgtggc  
4951 ctaactacggctacactagaaggacagtatttggatatctgcgctctgtctg  
5001 aagccagttaccttcggaaaaagagttggtagctctcttgatccggcaaac  
5051 aaccaccgctggtagcgggtgggttttttggtttgcaagcagcagattacgc  
5101 gcagaaaaaaaggatctcaagaagatccttttgatcttttctacggggtct  
5151 gacgctcagtggaacgaaaactcacgttaagggattttgggtcatgagatt  
5201 atcaaaaaggatcttcacctagatccttttaaatataaaatgaagtttta  
5251 aatcaatctaaagtatatatgagtaaacttggtctgacagttaccaatgc  
5301 ttaatcagtgaggcacctatctcagcgatctgtctatttctgttcacccat  
5351 agttgcctgactccccgtcgtgtagataactacgatacgggagggcttac  
5401 catctggccccagtgctgcaatgataccgcgagaccacgctcaccggct  
5451 ccagattttatcagcaataaaccagccagccggaagggccgagcgcagaag  
5501 tggctctgcaactttatccgectccatccagtcctattaattggttgcggg  
5551 aagctagagtaagtagttcgccagttaatagtttgcgcaacggttgttggc  
5601 attgtcacaggcatcgtggtgtcacgctcgtcgtttgggtatggcttcatt  
5651 cagctccggttcccaacgatcaaggcgagttacatgatcccccatgttgtt  
5701 gcaaaaaagcgggttagctccttcggctcctccgatcgttgtcagaagtaag  
5751 ttggccgcagtggttatcactcatggttatggcagcactgcataattctct  
5801 tactgtcatgccatccgtaagatgcttttctgtgactgggtgagtactcaa

FIGURE 8F

5851 ccaagtcattctgagaatagtgatatgcggcgaccgagttgctctttgcccg  
5901 gcgtaataacgggataataaccgcgccacatagcagaactttaaaagtgt  
5951 catcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgc  
6001 tgttgagatccagttcgatgtaaccactcgtgcacccaactgatcttca  
6051 gcctcttttactttcaccagcggtttctgggtgagcaaaacaggaaggca  
6101 aaatgccgcaaaaaaggaataaggggcacacggaaatggtgaatactca  
6151 tactcttcctttttcaatatattgaagcatttatcagggttattgtctc  
6201 atgagcggatacatatttgaatgtatttagaaaaataaacaatatggggt  
6251 tccgcgcacatttccccgaaaagtgccacct

FIGURE 9A

```

1  gacgtcgcgggcgctctagggcctccaaaaagcctcctcactacttctgg
51  aatagctcagaggccgagggcgccctcgccctctgcataataaaaaaaat
101 tagtcagccatgcatggggcggagaatgggcggaactgggcggagttagg
151 ggcgggatgggcggagttagggcgggactatgggttctgactaattgag
201 atgcatgctttgcatacttctgctgctggggagcctggggactttccac
251 acctgggttctgactaattgagatgcatgctttgcatacttctgctgct
301 ggggagcctggggactttccacaccctaactgacacacattccacagaat
351 taattcccggggatcgatccgctcgacgtacgactagttattaatagtaat
401 caattacggggctcattagttcatagcccatatattggagttccgcgttaca
451 taacttacggtaaatggcccgctggctgaccgccccacgacccccgcc
501 attgacgtcaataatgacgtatgttcccatagtaacgccaatagggactt
551 tccattgacgtcaatgggtggactatttacggtaaactgccacttggca
601 gtacatcaagtgtatcatatgccaaagtacgccccctattgacgtcaatga
651 cggtaaatggcccgctggcattatgccagttacatgaccttatgggact
701 ttctacttggcagttacatctacgtatttagtcatcgctattaccatgggtg
751 atgcgggttttggcagttacatcaatgggcgtggatagcgggtttgactcacg
801 gggatttccaagtctccacccattgacgtcaatgggagttttgtttggc
851 accaaaatcaacgggactttccaaaatgtcgttaacaactccgccccattg
901 acgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagc
                                     EcoRI
951  tgggtacgtgaaccgtcagatcgccctggagacgccatcgaattctgagca
1001 cacaggacctcaccatgggatggagctgtatcatcctcttcttggtagca
      M G W S C I I L F L V A
      Leader start

                                     SacI
1051 acagctacaggtgtccactccgagctcacgcagccgccctcagttctctgc
      T A T G V H S E L T Q --
      Processed N-term

```

FIGURE 9B

1101 ggccccaggacagaaggtcaccatctctctgcactgggagcagctccaacc  
1151 tgggggcagggttatgatgttcaactgggtaccggcaacttccagggcagacc  
1201 cccaaactctcatctatgataacaacaatcgccctcagggggtccctga  
1251 ccgattctctggctccaagtctggccctcagcctccctggccatctctg  
1301 ggctccaggctgaggatgaggctgattattactgccagtcctatgacagc  
1351 agcctgaatgggttatgtcttcggaactgggaccagctcaccgtcctagg  
AvrII  
T Q L T V L G  
Framework IV / Cλ  
1401 tcagcccaaggctgccccctcgggtcactctgttccccctctctgagg  
1451 agcttcaagccaacaaggccacactgggtgtgtctcataagtgcattctac  
1501 cggggagccgtgacagtgccctggaaggcaattagcagccccgtcaaggc  
1551 gggagtgaggaccaccacacccctccaaacaaagcaacaacaagtacggg  
1601 ccagcagctatctgagcctgacgctgagcagtggaagtccacagaagg  
1651 tacagctgccagggtcacgcatgaagggagcacccgtggagaagacagtggc  
1701 ccctacagaatgttcatagttcttagatctacgtatgatcagcctcgactg  
P T E C S \* C-term light chain  
1751 tgcttcttagttgccagccatctgttgtttgccccctccccgtgccttcc  
1801 ttgaccttggaaggtgccactcccactgtcctttcctaataaaaatgagga  
1851 aattgcacgcattgtgtctgagtaggtgtcattctattctgggggtgggg  
1901 tggggcaggacagcaagggggaggattgggaagacaatagcaggcatgct  
1951 ggggatgcggtgggtctctatggaaccagctggggctcgacagctcgagct  
2001 agcttttgcttctcaatttcttatttgcataatgagaaaaaaggaaaatt  
2051 aattttaacaccaattcagtagttgattgagcaaatgcgttgccaaaaag  
2101 gatgcttttagagacagtgttctctgcacagataaggacaaacattattca  
2151 gaggggagtaccagagctgagactcctaagccagtgagtggcacagcatt

FIGURE 9C

2201 ctaggggagaaatatgcttgtcatcaccgaagcctgattccgtagagccac  
2251 accttggtaagggccaatctgctcacacaggatagagagggcaggagcca  
2301 gggcagagcatataaaggtgaggtaggatcagttgtcctcacatttgctt  
2351 ctgacatagttgtgttgggagcttggatogatocaccatggttgaacaag  
2401 atggattgcacgcagggttctccggcgccttgggtggagaggtatttcggc  
2451 tatgactgggcacacaacagacaatcgggtgctctgatgccgcggtgttccg  
2501 gctgtcagcgcagggggcggcgttctttttgtcaagaccgacctgtccg  
2551 gtgccctgaatgaactgcaggacgaggcagcgggtatctgtggctggcc  
2601 acgacgggcgttcccttgccgagctgtgctcgacgttgtcactgaagcggg  
2651 aagggactggctgctatttgggcgaagtgcgggggcaggatctcctgtcat  
2701 ctacacctgtccttgcgagaaaagtatccatcatggctgatgcaatgcgg  
2751 cggctgcatacgccttgatccggctacctgcccattcgaccaccaagcgaa  
2801 acatcgcacatcgagcgcagcacgtactcggatggaagccggtcttctgcatc  
2851 aggatgatctggacgaagagcatcaggggctcgcgcagccgaactgttc  
2901 gccaggctcaaggcgcgcgtgcccgacggcgaggatctcgtcgtgaccca  
2951 tggcgatgcctgcttgcgcaatatcatgggtgaaaaatggccgcttttctg  
3001 gattcatcgactgtggccggctgggtgtggcggaccgctatcaggacata  
3051 gcgttggctacccgtgatatttctgaagagcttggcggcgcaatgggctga  
3101 ccgcttctcgtgctttacgggtatcgccgctcccgattcgcagcgcacg  
3151 ccttctatcgcttcttgcagagtcttcttgagcgggactctgggggttcg  
3201 aaatgaccgaccaagcgacgccccacctgccatcacgagatttcgattcc  
3251 accgccgccttctatgaaaggttgggcttcggaatcgttttccgggacgc  
3301 cggctggatgatcctccagcggggatctcatgctggagtcttctcgccc  
3351 accccaacttgtttattgcagcttataatgggttacaaataaagcaatagc



FIGURE 9D

3401 atcacaaatttcacaaataaagcatttttttactgcattcttagttgtgg  
3451 tttgtccaaactcatcaatgtatcttatcatgtctggatcgcggccgcga  
3501 tcccgtcgagagcttggcgtaaatcatggtcatagtctgttctctgtgtgaa  
3551 attgttatccgctcacaaattccacacacatacagaccggaagcataaag  
3601 tgtaaagcctggggtgectaatgagtgaagtaactcacattaattgcgtt  
3651 gcgctcactgcccgtttccagtcgggaaacctgtcgtgcccagctgcatt  
3701 aatgaatcggccaacgcgcggggagaggcggtttggcgtattgggcgctct  
3751 tccgcttctctcgctcactgactcgcctgcgctcggctcgttcggctgcggcg  
3801 agcggtatcagctcactcaaaggcggtaatacggttatccacagaatcag  
3851 gggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccagg  
3901 aaccgtaaaaaggccgcggttgctggcggtttttccataggtctcgcccccc  
3951 tgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaaccgga  
4001 caggactataaagataaccaggcggtttccccctggaagctccctcgtgcgc  
4051 tctctctgttccgaccctgccgcttaccggatacctgtccgcctttctccc  
4101 ttcgggaagcgtggcgctttctcaatgctcacgctgtaggatatctcagtt  
4151 cgggtgtaggctcgttcgctccaagctgggctgtgtgcacgaacccccggtt  
4201 cagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaaccc  
4251 ggtaagacacgacttatcgccactggcagcagccactggtaacaggatta  
4301 gcagagcgaggtatgtaggggtgctacagagttcttgaaagtggtggcct  
4351 aactacggctacactagaaggacagtatttggtatctgcgctctgctgaa  
4401 gccagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaa  
4451 ccaccgctggtagcgggtggttttttgtttgcaagcagcagattacgcgc  
4501 agaaaaaaaggatctcaagaagatcctttgatctttctacggggtctga  
4551 cgctcagtggaaacgaaaactcacggttaagggttttgggtcatgagattat

FIGURE 9E

4601 caaaaaggatcttccacctagatccttttaaatataaaatgaagttttaa  
4651 tcaatctaaagtatatatagagtaaaccttggctctgacagttaccaatgctt  
4701 aatcagtgaggcaccttatctcagcgatctgtctatttcggttcatccatag  
4751 ttgacctgactccccgctcggtgtagataactacgatacgggagggcctacca  
4801 tctggccccagtgctgcaatgataccgcgagacccacgctcaccggctcc  
4851 agattttatcagcaataaaccagccagccggaagggccgagcgcagaagtg  
4901 gtccctgcaactttatccgcctccatccagtcctattaattgttgccgggaa  
4951 gctagagtaagtagttcgccagttaatagtttgcgcaacggtgttgccat  
5001 tgctacaggcatcgtggtgtcacgctcgtcgtttggtatggcttcattca  
5051 gctccggttcccaacgatcaaggcgagttacatgatcccccatggtgtgc  
5101 aaaaaagcggtagctccttcggtcctccgatcgttgtcagaagtaagtt  
5151 ggccgcagtggtatcactcatggttatggcagcactgcataattctctta  
5201 ctgtcatgccatccgtaagatgcttttctgtgactggtgagtactcaacc  
5251 aagtcattctgagaatagtgtatgcggcgaccgagttgctcctgcccggc  
5301 gtcaatacgggataataaccgcgccacatagcagaactttaaaagtgtca  
5351 tcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgctg  
5401 ttgagatccagtttcgatgtaaccactcgtgcacccaactgatcttcagc  
5451 atcttttactttcaccagcgtttctgggtgagcaaaaaacaggaaggcaaa  
5501 atgccgcaaaaaagggaataaggcgacacggaaatgttgataactcata  
5551 ctcttcctttttcaatattattgaagcatttatcagggttattgtctcat  
5601 gagcggatacatatattgaaatgtatttagaaaaataacaaataggggttc  
5651 cgcgcacatttccccgaaaagtgccacct

FIGURE 10A

	EcoRI <u>gaattc</u> tgagca	1000
cacaggacctcaccatggg	atggagctgtatcatcctcttcttggtagca	1050
	M G W S C I I L F L V A	
acagctacaggtgtccactccgaggtg	cagctggaggagctctgggggagg	1100
T A T G V H S	E V Q L V E S -	
	N-term	
cttggtacagcctggggggtccctgagactctcctg	cgcagcctctggag	1150
tctccctcagtggtgatacaagatgaactgggtccgc	cagggtccaggggaag	1200
gggctggaatgggtctcttccattactggtatgagta	attacatacacta	1250
ctcagactcagtgaaagggcgattcaccatctccagag	acaacgccatga	1300
actcactgtatctgcaaatgaacagcctgacagccg	aggacacgggtgtt	1350
tattattgtg	gcacacaacccgggggagctggcgcccttttgaccattgggg	1400
	Bsp120I	
ccagggaaaccctgggtcaccgtctcctcagcctcc	acaaagggcccatcgg	1450
tcttccccctggcacccctcctccaagagcacctct	gggggcacagcggcc	1500
ctggggtgcctgggtcaaggactacttccccgaacc	gggtgacgggtgctcgtg	1550
gaactcaggcgccctgaccagcggcgtgcacacctt	cccggtgttcctac	1600
agtcctcaggactctactcctcagcagcgtggtgac	cgtgcctccagc	1650
agcttggggcaccagacctacatctgcaacgtgaat	cacaagccagcaa	1700
caccaaggtggacaagaaagttgagcccaaatctt	gtgacaaaactcaca	1750
catgcccaccgtgcccagcacctgaactcctggggg	gaccgtcagtccttc	1800
ctcttccccccaaaacccaaggacacctcatgatct	ccggaccctga	1850
ggtcacatgcgtgggtgggtggacgtgagccacga	agacctgagggtcaagt	1900
tcaactggtacgtggacggcgtggaggtgcataat	gccaagacaaagccg	1950
cgggaggagcagtagaacagcacgtaccgggtgg	tcagcgtcctcacctg	2000
cctgcaccaggactggctgaatggcaaggagtaca	agtgcaaggctctcca	2050

FIGURE 10B

acaaagccctcccagcccccatcgagaaaaccatctccaaagccaaaggg 2100  
cagccccgagaaccacaggtgtacacctgcccccatcccggaatgagct 2150  
gaccaagaaccaggtcagcctgacctgcctggcctcaaaggtctctatccca 2200  
gcgacatcgccgtggagtgaggagagcaatgggcagccggagaacaactac 2250  
aagaccacgcctcccggtgctggactccgacggctccttcttctctacag 2300  
caagctcacctgggacaagagcaggtggcagcaggggaacgtcttctctcat 2350  
gctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc 2400  
tcctgtctccgggtaaatgatagatatct  
S P G K \*

FIGURE 11

	EcoRI	
	<u>gaattc</u> tgagca	1000
cacaggacctcaccatgggatggagctgtatcatcctctcttggtagca		1050
	M G W S C I I L F L V A	
acagctacaggtgtccactcc <u>cagtcctgtt</u> gacgcagccgcctcagt		1100
	T A T G V H S <u>Q S V</u> L T Q -	
	N-term	
ctctgcggccccaggacagaaggtcaccatctcctgcactgggagcagct		1150
ccaacctcggggcaggttatgatgttcaactgggtaccggcaactccagggt		1200
acagcccccaactctcctatctatgataacaacaatcgccctcaggggt		1250
ccttgaccgattctctgggtccaagtctggccctcagcctccctggcca		1300
tctctgggctccaggctgaggatgaggctgattattactgccagtcctat		1350
gacagcagcctgaatgggttatgtcttcggaactgggaccagctcacgt		1400
AvrII		
<u>cctagg</u> tcagcccaaggctgccccctcggtcactctgttccgcctcct		1450
ctgaggagcttcaagccaacaaggccacactgggtgtctcataagtga		1500
ttctaccgggagccgtgacagtggtggaaggcaattagcagcccggt		1550
caaggcgggagtgagaccaccacacctccaaacaaagcaacaacaagt		1600
acgcgccagcagctatctgagcctgacgctgagcagtggaagtcacc		1650
agaaggtagcgtgccagggtcacgcatgaagggagcaccgtggagaagac		1700
agtggccctacagaatgttc <u>atag</u> ttctagatctacgtatgatcagcct		1750
	P T E C S *	

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: SmithKline Beecham, PLC
- (ii) TITLE OF INVENTION: Human Monoclonal Antibody
- (iii) NUMBER OF SEQUENCES: 21
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: SmithKline Beecham Corporation
  - (B) STREET: 709 Swedeland Road
  - (C) CITY: King of Prussia
  - (D) STATE: PA
  - (E) COUNTRY: USA
  - (F) ZIP: 19406-2799
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: GB
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: King, William T.
  - (B) REGISTRATION NUMBER: 30,954
  - (C) REFERENCE/DOCKET NUMBER: #
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 610-270-4800
  - (B) TELEFAX: 610-270-4026

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 336 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..336

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAG	TCT	GTG	TTG	ACG	CAG	CCG	CCC	TCA	GTC	TCT	GCG	GCC	CCA	GGA	CAG	48
Gln	Ser	Val	Leu	Thr	Gln	Pro	Pro	Ser	Val	Ser	Ala	Ala	Pro	Gly	Gln	
1				5					10					15		
AAG	GTC	ACC	ATC	TCC	TGC	ACT	GGG	AGC	AGC	TCC	AAC	CTC	GGG	GCA	GGT	96

Lys	Val	Thr	Ile	Ser	Cys	Thr	Gly	Ser	Ser	Ser	Asn	Leu	Gly	Ala	Gly		
			20					25					30				
TAT	GAT	GTT	CAC	TGG	TAC	CGG	CAA	CTT	CCA	GGG	ACA	GCC	CCC	AAA	CTC	144	
Tyr	Asp	Val	His	Trp	Tyr	Arg	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu		
		35				40					45						
CTC	ATC	TAT	GAT	AAC	AAC	AAT	CGG	CCC	TCA	GGG	GTC	CCT	GAC	CGA	TTC	192	
Leu	Ile	Tyr	Asp	Asn	Asn	Arg	Pro	Ser	Gly	Val	Pro	Asp	Arg	Phe			
	50				55					60							
TCT	GGC	TCC	AAG	TCT	GGC	CCC	TCA	GCC	TCC	CTG	GCC	ATC	TCT	GGG	CTC	240	
Ser	Gly	Ser	Lys	Ser	Gly	Pro	Ser	Ala	Ser	Leu	Ala	Ile	Ser	Gly	Leu		
	65				70				75					80			
CAG	GCT	GAG	GAT	GAG	GCT	GAT	TAT	TAC	TGC	CAG	TCC	TAT	GAC	AGC	AGC	288	
Gln	Ala	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ser	Tyr	Asp	Ser	Ser		
			85					90					95				
CTG	AAT	GGT	TAT	GTC	TTC	GGA	ACT	GGG	ACC	CAG	CTC	ACC	GTC	CTA	GGT	336	
Leu	Asn	Gly	Tyr	Val	Phe	Gly	Thr	Gly	Thr	Gln	Leu	Thr	Val	Leu	Gly		
			100				105						110				

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 112 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gln	Ser	Val	Leu	Thr	Gln	Pro	Pro	Ser	Val	Ser	Ala	Ala	Pro	Gly	Gln		
1				5				10					15				
Lys	Val	Thr	Ile	Ser	Cys	Thr	Gly	Ser	Ser	Ser	Asn	Leu	Gly	Ala	Gly		
		20					25						30				
Tyr	Asp	Val	His	Trp	Tyr	Arg	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu		
	35					40					45						
Leu	Ile	Tyr	Asp	Asn	Asn	Arg	Pro	Ser	Gly	Val	Pro	Asp	Arg	Phe			
	50				55				60								
Ser	Gly	Ser	Lys	Ser	Gly	Pro	Ser	Ala	Ser	Leu	Ala	Ile	Ser	Gly	Leu		
	65				70				75					80			
Gln	Ala	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ser	Tyr	Asp	Ser	Ser		
			85					90					95				
Leu	Asn	Gly	Tyr	Val	Phe	Gly	Thr	Gly	Thr	Gln	Leu	Thr	Val	Leu	Gly		
			100				105						110				

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 357 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG GGG	48
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly	
1 5 10 15	
TCC CTG AGA CTC TCC TGC GCA GCC TCT GGA GTC TCC CTC AGT GGA TAC	96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu Ser Gly Tyr	
20 25 30	
AAG ATG AAC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG GAA TGG GTC	144
Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	
35 40 45	
TCT TCC ATT ACT GGT ATG AGT AAT TAC ATA CAC TAC TCA GAC TCA GTG	192
Ser Ser Ile Thr Gly Met Ser Asn Tyr Ile His Tyr Ser Asp Ser Val	
50 55 60	
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAC GCC ATG AAC TCA CTG TAT	240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Met Asn Ser Leu Tyr	
65 70 75 80	
CTG CAA ATG AAC AGC CTG ACA GCC GAG GAC ACG GGT GTT TAT TAT TGT	288
Leu Gln Met Asn Ser Leu Thr Ala Glu Asp Thr Gly Val Tyr Tyr Cys	
85 90 95	
GCG ACA CAA CCG GGG GAG CTG GCG CCT TTT GAC CAT TGG GGC CAG GGA	336
Ala Thr Gln Pro Gly Glu Leu Ala Pro Phe Asp His Trp Gly Gln Gly	
100 105 110	
ACC CTG GTC ACC GTC TCC TCA	357
Thr Leu Val Thr Val Ser Ser	
115	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 119 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly	
1 5 10 15	
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu Ser Gly Tyr	
20 25 30	
Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	



35					40					45					
Ser	Ser	Ile	Thr	Gly	Met	Ser	Asn	Tyr	Ile	His	Tyr	Ser	Asp	Ser	Val
50					55					60					
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Met	Asn	Ser	Leu	Tyr
65					70					75					
Leu	Gln	Met	Asn	Ser	Leu	Thr	Ala	Glu	Asp	Thr	Gly	Val	Tyr	Tyr	Cys
85					90					95					
Ala	Thr	Gln	Pro	Gly	Glu	Leu	Ala	Pro	Phe	Asp	His	Trp	Gly	Gln	Gly
100					105					110					
Thr	Leu	Val	Thr	Val	Ser	Ser									
115															

(2) INFORMATION FOR SEO ID No:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

[illegible]

(2) INFORMATION FOR SEO ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1           5           10           15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20           25           30
Glu Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35           40           45
Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
          50           55           60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
          65           70           75           80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
          85           90           95
Ala Arg

```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 138 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1           5           10           15
Val His Ser Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
          20           25           30
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu
          35           40           45
Ser Gly Tyr Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
          50           55           60
Glu Trp Val Ser Ser Ile Thr Gly Met Ser Asn Tyr Ile His Tyr Ser
          65           70           75           80
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Met Asn
          85           90           95
Ser Leu Tyr Leu Gln Met Asn Ser Leu Thr Ala Glu Asp Thr Gly Val

```

100 105 110

Tyr Tyr Cys Ala Thr Gln Pro Gly Glu Leu Ala Pro Phe Asp His Trp  
115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
130 135

(2) INFORMATION FOR SEO ID NO:8:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 138 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Gly	Trp	Ser	Cys	Ile	Ile	Leu	Phe	Leu	Val	Ala	Thr	Ala	Thr	Gly
1			5						10					15	
Val	His	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln
			20					25					30		
Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Val	Ser	Leu
		35					40					45			
Ser	Gly	Tyr	Lys	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu
	50					55					60				
Glu	Trp	Val	Ser	Ser	Ile	Thr	Gly	Met	Ser	Asn	Tyr	Ile	His	Tyr	Ser
65					70					75					80
Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Met	Asn
			85						90					95	
Ser	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Thr	Ala	Glu	Asp	Thr	Gly	Val
			100					105					110		
Tyr	Tyr	Cys	Ala	Thr	Gln	Pro	Gly	Glu	Leu	Ala	Pro	Phe	Asp	His	Trp
		115					120					125			
Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser						
	130					135									

(2) INFORMATION FOR SEO ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 111 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1           5           10           15
Lys Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Leu Gly Ala Gly
          20           25           30
Tyr Asp Val His Trp Tyr Arg Gln Leu Pro Gly Thr Ala Pro Lys Leu
          35           40           45
Leu Ile Tyr Asp Asn Asn Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
          50           55           60
Ser Gly Ser Lys Ser Gly Pro Ser Ala Ser Leu Ala Ile Ser Gly Leu
          65           70           75           80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
          85           90           95
Leu Asn Gly Tyr Val Phe Gly Thr Gly Thr Gln Leu Thr Val Leu
          100          105          110
```

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 90 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1           5           10           15
Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
          20           25           30
Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
          35           40           45
Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
          50           55           60
Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
          65           70           75           80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys
          85           90
```

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 128 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1           5           10           15
Val His Ser Glu Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly
          20           25           30
Gln Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala
          35           40           45
Gly Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys
          50           55           60
Leu Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg
          65           70           75           80
Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly
          85           90           95
Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser
          100          105          110
Ser Leu Asn Gly Tyr Val Phe Gly Thr Gly Thr Gln Leu Thr Val Leu
          115          120          125

```

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 130 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1           5           10           15
Val His Ser Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala
          20           25           30
Pro Gly Gln Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile
          35           40           45
Gly Ala Gly Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala
          50           55           60
Pro Lys Leu Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro
          65           70           75           80

```

GACGTCGCGG	CCGCTCTAGG	CCTCCAAAAA	AGCCTCCTCA	CTACTTCTGG	AATAGCTCAG	60
AGGCCGAGGC	GGCCTCGGCC	TCTGCATAAA	TAAAAAAAT	TAGTCAGCCA	TGCATGGGGC	120
GGAGAATGGG	CGGAAC TGGG	CGGAGTTAGG	GGCGGGATGG	GCGGAGTTAG	GGGCGGGACT	180
ATGGTTGCTG	ACTAATTGAG	ATGCATGCTT	TGCATACTTC	TGCCTGCTGG	GGAGCCTGGG	240
GACTTTCCAC	ACCTGGTTGC	TGACTAATTG	AGATGCATGC	TTTGCACTAT	TC TGCCTGCT	300
GGGGAGCCTG	GGGACTTTCC	ACACCCTAAC	TGACACACAT	TCCACAGAAT	TAATTCCCGG	360
GGATCGATCC	GTGCGAGTAC	GACTAGTTAT	TAATAGTAAT	CAATTACGGG	GTCATTAGTT	420
CATAGCCCCAT	ATATGGAGTT	CCGCGTTACA	TAACTTACGG	TAAATGGCCC	GCCTGGCTGA	480
CCGCCCAACG	ACCCCCGCCC	ATTGACGTCA	ATAATGACGT	ATGTTCCCAT	AGTAACGCCA	540
ATAGGGACTT	TCCATTGACG	TCAATGGGTG	GACTATTTCAC	GGTAAACTGC	CCACTTGCCA	600
GTACATCAAG	TGTATCATAT	GCCAAGTACG	CCCCCTATTG	ACGTCAATGA	CGGTAAATGG	660
CCCGCTGGC	ATTATGCCCA	GTACATGACC	TTATGGGACT	TTCCTACTTG	GCAGTACATC	720
TACGTATTAG	TCATCGCTAT	TACCATGGTG	ATGCGGTTTT	GGCAGTACAT	CAATGGGCGT	780
GGATAGCGGT	TTGACTCACG	GGGATTTCCA	AGTCTCCACC	CCATTGACGT	CAATGGGAGT	840
TTGTTTTGGC	ACCAAAATCA	ACGGGACTTT	CCAAAAATGTC	GTAACAACTC	CGCCCCATTG	900
ACGCAAAATGG	GCGGTAGGCG	TGTACGGTGG	GAGGTCTATA	TAAGCAGAGC	TGGGTACGTG	960
AACCGTCAGA	TCGCCTGGAG	ACGCCATCGA	ATTCTGAGCA	CACAGGACCT	CACCATGGGA	1020
TGGAGCTGTA	TCATCTCTTT	CTTGGTAGCA	ACAGCTACAG	GTGTCCACTC	CGAGGTCCAA	1080

CTGCTCGAGT	CTGGGGGAGG	CTTGGTACAG	CCTGGGGGGT	CCCTGAGACT	CCTCTGCGCA	1140
GCCTCTGGAG	TCTCCCTCAG	TGGATACAAG	ATGAACCTGG	TCCGCCAGGC	TCCAGGGGAAG	1200
GGGCTGGAAT	GGGTCTCTTC	CATTACTGGT	ATGAGTAATT	ACATACACTA	CTCAGACTCA	1260
GTGAAGGGCC	GATTCAACAT	CTCCAGAGAC	AACGCCATGA	ACTCACTGTA	TCTGCAATATG	1320
AACAGCCTGA	CAGCCGAGGA	CACGGGTGTT	TATTATTGTG	CGACACAACC	GGGGGAGCTG	1380
GCGCCTTTTG	ACCATTGGGG	CCAGGGAACC	CTGGTCACCG	TCTCTCAGC	CTCCACCAAG	1440
GGCCCATCGG	TCTTCCCCCT	GGCACCCCTC	TCCAAGAGCA	CCTCTGGGGG	CACAGCGGCC	1500
CTGGGCTGCC	TGGTCAAGGA	CTACTTCCCC	GAACCGGTGA	CGGTGTCGTG	GAACCTCAGG	1560
GCCCTGACCA	GCGGCGTGCA	CACCTTCCCG	GCTGTCTTAC	AGTCTCAGG	ACTCTACTCTC	1620
CTCAGCAGCG	TGGTGACCGT	GCCCTCCAGC	AGCTTGGGCA	CCCAGACCTA	CATCTGCAAC	1680
GTGAATCACA	AGCCCAGCAA	CACCAAGGTG	GACAAGAAAG	TTGAGCCCAA	ATCTTGTGAC	1740
AAAACCTACA	CATGCCCAAC	GTGCCCAGCA	CCTGAACCTC	TGGGGGGACC	GTCAGTCTTC	1800
CTCTTCCCCC	CAAAACCCAA	GGACACCCCT	ATGATCTCCC	GGACCCCTGA	GGTCACATGC	1860
GTGGTGGTGG	ACGTGAGCCA	CGAAGACCCT	GAGGTCAAGT	TCAACTGGTA	CGTGGACGGC	1920
GTGGAGGTGC	ATAATGCCAA	GACAAAGCCG	CGGGAGGAGC	AGTACAACAG	CACGTACCGG	1980
GTGGTCAGCG	TCCTCACCGT	CCTGCACCAG	GACTTGGCTG	ATGGCAAGGA	GTACAAGTGC	2040
AAGGTCTCCA	ACAAAGCCCT	CCCAGCCCCC	ATCGAGAAAA	CCATCTCCAA	AGCCAAAGGG	2100
CAGCCCCGAG	AACCACAGGT	GTACACCCCT	CCCCCATCCC	GGGATGAGCT	GACCAAGAAC	2160
CAGGTGACGC	TGACCTGCCT	GGTCAAAGGC	TTCATATCCA	GCGACATCGC	CGTGGAGTGG	2220
GAGAGCAATG	GGCAGCCGGA	GAACAACCTA	AAGACCACGC	CTCCCGTGCT	GGACTCCGAC	2280
GGCTCCTTCT	TCCTCTACAG	CAAGCTCACC	GTGGACAAGA	GCAGGTGGCA	GCAGGGGAAC	2340
GTCTTCTCAT	GCTCCGTGAT	GCATGAGGCT	CTGCACAACC	ACTACACGCA	GAAGAGCCTC	2400
TCCCTGTCTC	CGGGTAAATG	ATAGATATCT	ACGTATGATC	AGCCTCGACT	GTGCCTTCTA	2460
GTTGCCAGCC	ATCTGTGTGT	TGCCCCCTCC	CCGTGCCTTC	CTTGACCCTG	GAAGGTGCCA	2520
CTCCCACTGT	CCTTTCCTAA	TAAATGAGG	AAATTGCATC	GCATTGTCTG	AGTAGGTGTC	2580
ATTCTATTCT	GGGGGGTGGG	GTGGGGCAGG	ACAGCAAGGG	GGAGGATTGG	GAAGACAATA	2640
GCAGGCATGC	TGGGGATGCG	GTGGGCTCTA	TGGAACCAGC	TGGGGCTCGA	CAGCGCTGGA	2700
TCTCCCGATC	CCCAGCTTTG	CTTCTCAATT	TCTTATTTGC	ATAATGAGAA	AAAAAGGAAA	2760
ATTAATTTTA	ACACCAATTC	AGTAGTTGAT	TGAGCAAATG	CGTTGCCAAA	AAGGATGCTT	2820
TAGAGACAGT	GTCTCTGCA	CAGATAAGGA	CAAACATTAT	TCAGAGGGAG	TACCCAGAGC	2880
TGAGACTCCT	AAGCCAGTGA	GTGGCACAGC	ATTCTAGGGA	GAAATATGCT	TGTCTATCAC	2940
GAAGCCTGAT	TCCGTAGAGC	CACACCTTGG	TAAGGGCCAA	TCTGCTCACA	CAGGATAGAG	3000

AGGGCAGGAG	CCAGGGCAGA	GCATATAAGG	TGAGGTAGGA	TCACTTGCTC	CTCACATTTC	3060
CTTCTGACAT	AGTTGTGTGG	GGAGCTTGGG	TAGCTTGGAC	AGCTCAGGGC	TGCGATTTCG	3120
CGCCAAACTT	GACGGCAATC	CTAGCGTGAA	GGCTGGTAGG	ATTTTATCCC	CGCTGCCATC	3180
ATGGTTTCGAC	CATTGAACTG	CATCGTCGCC	GTGTCCCAAA	ATATGGGGAT	TGGCAAGAAC	3240
GGAGACCTAC	CCTGGCCTCC	GCTCAGGAAC	GAGTTCAAGT	ACTTCCAAGG	AATGACCACA	3300
ACCTCTTCAG	TGGAAGGTAA	ACAGAATCTG	GTGATTATGG	GTAGGAAAAC	CTGGTTCTCC	3360
ATTCCTGAGA	AGAATCGACC	TTTAAAGGAC	AGAATTAATA	TAGTTCTCAG	TAGAGAACTC	3420
AAAGAACCAC	CACGAGGAGC	TCATTTTCTT	GCCAAAAGTT	TGGATGATGC	CTTAAGACTT	3480
ATTGAACAAC	CGGAATTGGC	AAGTAAAGTA	GACATGGTTT	GGATAGTCGG	AGGCAGTTCT	3540
GTTTACCAGG	AAGCCATGAA	TCAACCAGGC	CACCTTAGAC	TCTTTGTGAC	AAGGATCATG	3600
CAGGAATTTC	AAAGTGACAC	GTTTTCCTCA	GAAATTGATT	TGGGGAAATA	TAAACTTCTC	3660
CCAGAATACC	CAGGCGTCTC	CTCTGAGGTC	CAGGAGGAAA	AAGGCATCAA	GTATAAGTTT	3720
GAAGTCTACG	AGAAGAAAGA	CTAACAGGAA	GATGCTTTCA	AGTTCTCTGC	TCCCTCTCTA	3780
AAGCTATGCA	TTTTTATAAG	ACCATGGGAC	TTTTGCTGGC	TTTAGATCAG	CCTCGACTGT	3840
GCCTTCTAGT	TGCCAGCCAT	CTGTGTGTTG	CCCCCTCCCC	GTGCCTTCTC	TGACCTCGGA	3900
AGGTGCCACT	CCCAGTGTCC	TTTCTTAATA	AAATGAGGAA	ATTGCATCGC	ATTGCTGAGG	3960
TAGGTGTGAT	TCTATTCTGG	GGGGTGGGGT	GGGGCAGGAC	AGCAAGGGGG	AGGATTGGGA	4020
AGACAATAGC	AGGCATGCTG	GGGATGCGGT	GGGCTCTATG	GAACCAAGCTG	GGGCTCGATC	4080
GAGTGTATGA	CTGCGGCCGC	GATCCCGTCG	AGAGCTTGGC	GTAATCATGG	TCATAGCTGT	4140
TTCTCTGTGT	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	CATACGAGCC	GGAAGCATAA	4200
AGTGTAAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC	ATTAATTGCG	TTGCGCTCAC	4260
TGCCCCGCTT	CCAGTCGGGA	AACCTGTCTG	GCCAGCTGCA	TTAATGAATC	GGCCAAACGG	4320
CGGGGAGAGG	CGGTTTGCGT	ATTGGGCGCT	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	4380
GCTCGGTCTG	TCGGCTGCGG	CGAGCGGTAT	CAGCTCACTC	AAAGCGGTA	ATACGGTTAT	4440
CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	AAAAGGCCAG	AAAAGGCCCA	4500
GGAACCGTAA	AAAGCCCGCG	TTGCTGCGGT	TTTTCATAG	GCTCCGCCCC	CCTGACGAGC	4560
ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT	GGCGAAACCC	GACAGGACTA	TAAAGATACC	4620
AGGCGTTTCC	CCCTGGAAGC	TCCCTCGTGC	GCTCTCCTGT	TCCGACCCCTG	CCGCTTACCG	4680
GATACCTGTC	CGCTTTCTCT	CCTTCGGGAA	GCGTGCGCGT	TTCTCAATGC	TCACGCTGTA	4740
GGTATCTCAG	TTGCGGTAG	GTCGTTCTGCT	CCAAGCTGGG	CTGTGTGCAC	GAACCCCCCG	4800
TTACGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT	TGAGTCCAAC	CCGGTAAGAC	4860



ACGACTTATC	GCCACTGGCA	GCAGCCACTG	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	4920
GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC	CTAACTACGG	CTACACTAGA	AGGACAGTAT	4980
TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT	5040
CCGGCAAAAC	AACCACCGCT	GGTAGCGGTG	GTTTCTTTTGT	TTGCAAGCAG	CAGATTACGC	5100
GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	TACGGGGTCT	GACGCTCAGT	5160
GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT	ATCAAAAAGG	ATCTTCACCT	5220
AGATCCTTTT	AAATTAATAA	TGAAGTTTAA	AATCAATCTA	AAGTATATAT	GAGTAAACTT	5280
GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	CTCAGCGATC	TGCTTATTTT	5340
GTTTCATCCAT	AGTTGCTCTG	CTCCCCGTCG	TGTAGATAAC	TACGATACGG	GAGGGCTTAC	5400
CATCTGGCCC	CAGTGTCTGA	ATGATACCGC	GAGACCCACG	CTCACCGGCT	CCAGATTTAT	5460
CAGCAATAAA	CCAGCCAGCG	GGAAGGGCCG	AGCGCAGAAG	TGGTCTTGCA	ACTTTATCCG	5520
CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCT	CCAGTTAATA	5580
GTTTGCGCAA	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	GTACGCTCG	TCGTTTGATA	5640
TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	TACATGATCC	CCCATGTTGT	5700
GCAAAAAAAG	GGTTAGCTCC	TTCGGTCCTC	CGATCGTTGT	CAGAAGTAAG	TTGGCCGCAG	5760
TGTTATCACT	CATGGTTATG	GCAGCACTGC	ATAATTCTCT	TACTGTCTAT	CCATCCGTAA	5820
GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG	TGTATGCGGC	5880
GACCGAGTTG	CTCTTGCCCG	GCGTCAATAC	GGGATAATAC	CGCGCCACAT	AGCAGAACTT	5940
TAAAAGTGCT	CATCATTGGA	AAACGTTCTT	CGGGCGGAAA	ACTCTCAAGG	ATCTTACCGC	6000
TGTTGAGATC	CAGTTCGATG	TAACCCACTC	GTGCACCCAA	CTGATCTTCA	GCATCTTTTA	6060
CTTTCACCA	CGTTTCTGGG	TGAGCAAAAA	CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA	6120
TAAGGGCGAC	ACGGAATGTG	TGAATACTCA	TACTCTTCTT	TTTTCATAT	TATTGAAGCA	6180
TTTATCAGGG	TTATTGTCTC	ATGAGCGGAT	ACATATTTGA	ATGATTTTAG	AAAAATAAAC	6240
AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	AAGTGCCACC	T		6281

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5679 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GACGTCGCGG	CCGCTCTAGG	CCTCCAAAAA	AGCCTCCTCA	CTACTTCTGG	AATAGCTCAG	60
AGGCCGAGGC	GGCCTCGGCC	TCTGCATAAA	TAAAAAAAAT	TAGTCAGCCA	TGCATGGGGC	120
GGAGAATGGG	CGGAAC TGCG	CGGAGTTAGG	GGCGGGATGG	GCGGAGTTAG	GGGCGGGACT	180
ATGGTTGCTG	ACTAATTGAG	ATGCATGCTT	TGCATACTTC	TGCCTGCTGG	GGAGCCTGGG	240
GACTTTCCAC	ACCTGGTTGC	TGACTAATTG	AGATGCATGC	TTTGCATACT	TCTGCTCTGT	300
GGGGAGCCTG	GGGACTTTCC	ACACCCTAAC	TGACACACAT	TCCACAGAAT	TAATTCCC GG	360
GGATCGATCC	GTGACGTAC	GACTAGTTAT	TAATAGTAAT	CAATTACGGG	GTCATTAGTT	420
CATAGCCCAT	ATATGGAGTT	CCGCGTTACA	TAACCTACGG	TAAATGGCCC	GCCTGGCTGA	480
CCGCCCAACG	ACCCCCGCCC	ATTGACGTCA	ATAATGACGT	ATGTTCCCAT	AGTAACGCCA	540
ATAGGGACTT	TCCATTGACG	TCAATGGGTG	GACTATTTCAC	GGTAAACTGC	CCACTTGCCA	600
GTACATCAAG	TGTATCATAT	GCCAAGTACG	CCCCCTATTG	ACGTCAATGA	CGGTAAATGG	660
CCCGCCTGGC	ATTATGCCCA	GTACATGACC	TTATGGGACT	TTCCTACTTG	GCAGTACATC	720
TACGTATTAG	TCATCGCTAT	TACCATGGTG	ATGCGGTTTT	GGCAGTACAT	CAATGGGCGT	780
GGATAGCGGT	TTGACTCAGC	GGGATTTCAC	AGTCTCCACC	CCATTGACGT	CAATGGGAGT	840
TTGTTTGGC	ACCAAAATCA	ACGGGACTTT	CCAAATGTGC	GTAACAACTC	CGCCCCATTG	900
ACGCAAAATGG	GCGGTAGCGC	TGTACGGTGG	GAGGTCTATA	TAAGCAGAGC	TGGGTACGTG	960
AACCGTCAGA	TCGCTTGAGG	ACGCCATCGA	ATTCTGAGCA	CACAGGACCT	CACCATGGGA	1020
TGGAGCTGTA	TCATCTCTTT	CTTGGTAGCA	ACAGCTACAG	GTGTCCACTC	CGAGCTCACG	1080
CAGCCGCCCT	CAGTCTCTGC	GGCCCCAGGA	CAGAAGGTCA	CCATCTCCTG	CCTGGGAGC	1140
AGCTCCAACC	TCGGGGCAGG	TTATGATGTT	CACTGGTACC	GGCAACTTCC	AGGGACAGCC	1200
CCCAAACTCC	TCATCTATGA	TAACAACAAT	CGGCCCTCAG	GGGTCCCTGA	CCGATTCTCT	1260
GGCTCCAAGT	CTGGCCCCCTC	AGCCTCCCTG	GCCATCTCTG	GGCTCCAGGC	TGAGGATGAG	1320
GCTGATTATT	ACTGCCAGTC	CTATGACAGC	AGCCTGAATG	GTTATGTCTT	CGGAAC TGCG	1380
ACCCAGCTCA	CCGTCTTAGG	TCAGCCCCAAG	GCTGCCCCCT	CGGTCACTCT	GTTCCCCGCC	1440
TCCTCTGAGG	AGCTTCAAGC	CAACAAGGCC	ACACTGGTGT	GTCTCATAAG	TGACTTCTAC	1500
CCGGAGAGCG	TGACAGTGGC	CTGGAAGGCA	ATTAGCAGCC	CCGTCAAGGC	GGGAGTGGAG	1560
ACCACCACAC	CCTCCAAACA	AAGCAACAAC	AAGTACGCGG	CCAGCAGCTA	TCTGAGCCTG	1620
ACGCCCTGAG	AGTGGAAGTC	CCACAGAAGG	TACAGCTGCC	AGGTCAACGA	TGAAGGGAGC	1680
ACCGTGGAGA	AGACAGTGGC	CCCTACAGAA	TGTTTATAGT	TCTAGATCTA	CGTATGATCA	1740
GCCTCGACTG	TGCTTCTTAG	TTGCCAGCCA	TCTGTGTGTT	GCCTCTCCCC	CGTGCTTCTC	1800
TTGACCTCTG	AAGGTGCCAC	TCCCACTGTC	CTTCTCTAAT	AAAATGAGGA	AATTGCATCG	1860
CATTGTCTGA	GTAGGTGTCA	TTCTATTCTG	GGGGGTGGGG	TGGGGCAGGA	CAGCAAGGGG	1920

GAGGATTGGG	AAGACAATAG	CAGGCATGCT	GGGGATGCGG	TGGGCTCTAT	GGAACCAGCT	1980
GGGGCTCGAC	AGCTCGAGCT	AGCTTTGCTT	CTCAATTTC	TATTTGCATA	ATGAGAAAAA	2040
AAGGAAAAAT	AATTTTAAAC	CCAATTCAGT	AGTTGATTGA	GCAAAATGCGT	TGCCAAAAAG	2100
GATGCTTTAG	AGACAGTGT	CTCTGCACAG	ATAAGGACAA	ACATTATTCA	GAGGGAGTAC	2160
CCAGAGCTGA	GACTCCTAAG	CCAGTGAGTG	GCACAGCATT	CTAGGGAGAA	ATATGCTTGT	2220
CATCACCAGAA	GCCTGATTCC	GTAGAGCCAC	ACCTTGGTAA	GGGCCAATCT	GCTCACACAG	2280
GATAGAGAGG	GAGGAGCCCA	GGGCAGAGCA	TATAAGGTGA	GGTAGGATCA	GTTGCTCCTC	2340
ACATTGCTT	CTGACATAGT	TGTGTTGGGA	GCTTGGATCG	ATCCACCATG	GTTGAACAAAG	2400
ATGGATTGCA	CGCAGGTTCT	CCGGCCGCTT	GGGTGGAGAG	GCTATTCCGC	TATGACTGGG	2460
CACAACAGAC	AATCGGCTGC	TCTGATGCCG	CCGTGTTCCG	GCTGTCAGCG	CAGGGGCGCC	2520
CGGTTCTTTT	TGTCAAGACC	GACCTGTCCG	GTGCCCTGAA	TGAACTGCAG	GACGAGGCAG	2580
CGCGGCTATC	GTGGCTGGCC	ACGACGGGCG	TTCTTGC	AGCTGTGCTC	GACGTTGTCA	2640
CTGAAGCGGG	AAGGGACTGG	CTGCTATTGG	GCGAAGTGCC	GGGGCAGGAT	CTCCTGTCTAT	2700
CTCACCTTGC	TCCTGCCGAG	AAAGTATCCA	TCATGGCTGA	TGCAATGCGG	CGGCTGCATA	2760
CGCTTGATCC	GGCTACCTGC	CCATTGAGCC	ACCAAGCGAA	ACATCGCATC	GAGCGAGCAC	2820
GTACTCGGAT	GGAAGCCGGT	CTGTGCGATC	AGGATGATCT	GGACGAAGAG	CATCAGGGGC	2880
TCGCGCCAGC	CGAATGTTTC	GCCAGGCTCA	AGGCGCGCAT	GCCCAGCGGC	GAGGATCTCG	2940
TCGTGACCCA	TGGCGATGCC	TGCTTGCCGA	ATATCATGGT	GGAAAATGGC	CGCTTTTCTG	3000
GATTTCATCGA	CTGTGGCCGG	CTGGGTGTGG	CGGACCGCTA	TCAGGACATA	CGCTTGGCTA	3060
CCCGTGATAT	TGCTGAAGAG	CTTGGCGGCG	AATGGGCTGA	CCGCTTCTC	GTGCTTTACG	3120
GTATCGCGCG	TCCCGATTCC	CAGCGCATCG	CCTTCTATCG	CCTTCTTGAC	GAGTCTTCT	3180
GAGCGGGACT	CTGGGGTTCC	AAATGACCGA	CCAAGCGACG	CCCAACCTGC	CATCACGAGA	3240
TTTCGATTCC	ACCGCCCGCT	TCTATGAAAG	GTTGGGCTTC	GGAATCGTTT	TCCGGGACGC	3300
CGGCTGGATG	ATCCTCCAGC	GCGGGGATCT	CATGCTGGAG	TTCTTCGCCC	ACCCCAACTT	3360
GTTTATTGCA	GCTTATAATG	GTTACAAATA	AAGCAATAGC	ATCACAAATT	TCACAAATAA	3420
AGCATTTTTT	TCATGCAATT	CTAGTTGTGG	TTTGTCCAAA	CTCATCAATG	TATCTTATCA	3480
TGCTTGATC	GCGGCCGCGA	TCCCGTCGAG	AGCTTGGCGT	AATCATGGTC	ATAGCTGTTT	3540
CCTGTGTGAA	ATGTATTATC	GCTCACAAAT	CCACACAACA	TACGAGCCGG	AAGCATAAAG	3600
TGTAAAGCCT	GGGGTGCCCTA	ATGAGTGAGC	TAACACACAT	TAATTGCGTT	GCGCTCACTG	3660
CCCGCTTTTC	AGTCGGGAAA	CCTGTGCTGC	CAGCTGCATT	AATGAATCGG	CCAACGCGCG	3720
GGGAGAGGCG	GTTTGCGTAT	TGGGCGCTCT	TCCGCTTCCT	CGCTCACTGA	CTCGCTGCGC	3780

TCGGTCGTTTC	GGCTGCGGCG	AGCGGTATCA	GCTCACTCAA	AGGCGGTAAT	ACGGTTATCC	3840
ACAGAATCAG	GGGATAACGC	AGGAAAGAAC	ATGTGAGCAA	AAGGCCAGCA	AAAGGCCAGG	3900
AACCGTAAAA	AGGCCGCGTT	GCTGGCGTTT	TTCATAGGC	TCCGCCCCCC	TGACGAGCAT	3960
CACAAAAATC	GACGCTCAAG	TCAGAGGTGG	CGAAACCCGA	CAGGACTATA	AAGATACCAG	4020
CGGTTTCCCC	CTGGAAGCTC	CCTCGTGCGC	TCTCCTGTTC	CGACCTTGCC	GCTTACCGGA	4080
TACCTGTCCG	CCTTTCCTCC	TTCGGGAAGC	GTGGCGCTTT	CTCAATGCTC	ACGCTGTAGG	4140
TATCTCAGTT	CGGTGTAGGT	CCTTCGCTCC	AAGCTGGGCT	GTGTGCACGA	ACCCCCCGTT	4200
CAGCCCGACC	GCTGCGCCTT	ATCCGGTAAC	TATCGTCTTG	AGTCCAACCC	GGTAAGACAC	4260
GACTTATCGC	CACTGGCAGC	AGCCACTGGT	AACAGGATTA	GCAGAGCGAG	GTATGTAGGC	4320
GGTGCTACAG	AGTTCTTGAA	GTGGTGGCCT	AACACGGCT	ACACTAGAAG	GACAGTATTT	4380
GGTATCTGCG	CTCTGCTGAA	GCCAGTTACC	TTCGGA AAAA	GAGTTGGTAG	CTCTTGATCC	4440
GGCAAAACAA	CCACCGCTGG	TAGCGGTGGT	TTTTTTTGTT	GCAAGCAGCA	GATTACGCGC	4500
AGAAAAAAG	GATCTCAAGA	AGATCCTTTG	ATCTTTTCTA	CGGGGTCTGA	CGCTCAGTGG	4560
AACGAAAAC	CACGTTAAGG	GATTTTGGTC	ATGAGATTAT	CAAAAAGGAT	CTTCACCTAG	4620
ATCCTTTTAA	ATTAAAAATG	AAGTTTAA	TCAATCTAAA	GTATATATGA	GTAAACTTGG	4680
TCTGACAGTT	ACCAATGCTT	AATCAGTGAG	GCACCTATCT	CAGCGATCTG	TCTATTTCGT	4740
TCATCCATAG	TTGCCTGACT	CCCCGTCGTG	TAGATACTA	CGATACGGGA	GGGCTTACCA	4800
TCTGGCCCCA	GTGCTGCAAT	GATACCGCGA	GACCCACGCT	CACCGGCTCC	AGATTTATCA	4860
GCAATAAAC	AGCCAGCCGG	AAGGGCCGAG	CGCAGAAGTG	GTCTTGCAAC	TTTATCCGCC	4920
TCCATCCAGT	CTATTAATTG	TTGCCGGGAA	GCTAGAGTAA	GTAGTTGCC	AGTTAATAGT	4980
TTGCGCAACG	TTGTTGCCAT	TGCTACAGGC	ATCGTGGTGT	CACGCTCGTC	GTTTGGTATG	5040
GCTTCATCA	GCTCCGGTTC	CCAACGATCA	AGGCGAGTTA	CATGATCCCC	CATGTTGTGC	5100
AAAAAAGCGG	TTAGCTCCTT	CGGTCCCTCCG	ATCGTTGTCA	GAAGTAAGTT	GGCCGCAGTG	5160
TTATCACTCA	TGGTTATGGC	AGCACTGCAT	AATTCTCTTA	CTGTGATGCC	ATCCGTAAGA	5220
TGCTTTTCTG	TGACTGGTGA	GTACTCAACC	AAGTCATTCT	GAGAATAGTG	TATGCGGCGA	5280
CCGAGTTGCT	CTTGCCCGGC	GTCAATACGG	GATAATACCG	CGCCACATAG	CAGAACTTTA	5340
AAAGTGCTCA	TCATTGGA	ACGTTCTTCG	GGCGAAAAAC	TCTCAAGGAT	CTTACCGCTG	5400
TTGAGATCCA	GTTCGATGTA	ACCCACTCGT	GCACCCAACT	GATCTTCAGC	ATCTTTTACT	5460
TTCAACAGCG	TTTCTGGGTG	AGCAAAAAACA	GGAAGGCAAA	ATGCCGCAAA	AAAGGGAATA	5520
AGGGCGACAC	GGAAATGTTG	AATACTCATA	CTCTTCCTTT	TTCAATATTA	TTGAAGCATT	5580
TATCAGGGTT	ATTGTCTCAT	GAGCGGATAC	ATATTTGAAT	GTATTTAGAA	AAATAAACAA	5640
ATAGGGGTTT	CGCGCACATT	TCCCCGAAAA	GTGCCACCT			5679

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1442 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAATTCTGAG CACACAGGAC CTCACCATGG GATGGAGCTG TATCATCCTC TTCTTGGTAG	60
CAACAGCTAC AGGTGTCCAC TCCGAGGTGC AGCTGGTGA GTCTGGGGGA GGCTTGGTAC	120
AGCCTGGGGG GTCCCTGAGA CTCTCCTGCG CAGCCTCTGG AGTCTCCCTC AGTGGATACA	180
AGATGAACTG GGTCCGCCAG GCTCCAGGGA AGGGGCTGGA ATGGGTCTCT TCCATTACTG	240
GTATGAGTAA TTACATACAC TACTCAGACT CAGTGAAGGG CCGATTACAC ATCTCCAGAG	300
ACAACGCCAT GAACTCACTG TATCTGCAA TGAACAGCCT GACAGCCGAG GACACGGGTG	360
TTTATTATTG TGCACACAA CCGGGGGAGC TGGCGCCTTT TGACCATTTG GGCCAGGGAA	420
CCCTGGTCAC CGTCTCCTCA GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCCT	480
CCTCCAAGAG CACCTCTGGG GGCACAGCGG CCCTGGGCTG CTTGGTCAAG GACTACTTCC	540
CCGAACCGGT GACGGTGTG TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC	600
CGGCTGTCTT ACAGTCTCTA GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA	660
GCAGCTTGGG CACCCAGACC TACATCTGCA ACGTGAATCA CAAGCCAGC AACACCAAGG	720
TGGACAAGAA AGTTGAGCCC AAATCTTGTG ACAAAACTCA CACATGCCCA CCGTGCCAG	780
CACCTGAACT CTTGGGGGGA CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC	840
TCATGATCTC CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC	900
CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC	960
CGCGGGAGGA GCAGTACAAC AGCACGTACC GGGTGGTCAG CGTCTCACC GTCTGTCACC	1020
AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCTCAGCC	1080
CCATCAGAAA AACCATCTCC AAGGCCAAG GGCAGCCCCG AGAACCACAG GTGTACACCC	1140
TGCCCCATC CCGGGATGAG CTGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG	1200
GCTTCTATCC CAGCGCATC GCCGTGGAGT GGGGAGAGCA TGGGCGAGCG GAGAACAAC	1260
ACAAGACCAC GCCTCCCGTG CTGGAATCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA	1320
CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG	1380
CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA TGATAGATAT	1440

CT

1442

## (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 762 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GAATTCTGAG CACACAGGAC CTCACCATGG GATGGAGCTG TATCATCCTC TTCTTGGTAG	60
CAACAGCTAC AGGTGTCCAC TCCAGTCTG TGTGACGCA GCCGCCCTCA GTCTCTGCGG	120
CCCCAGGACA GAAGTCCACC ATCTCCTGCA CTGGGAGCAG CTCCAACCTC GGGGCAGGTT	180
ATGATGTTCA CTGGTACCGG CAACTTCCAG GGACAGCCCC CAAACTCCTC ATCTATGATA	240
ACAACAATCG GCCCTCAGGG GTCCCTGACC GATTCTCTGG CTCCAAGTCT GGCCCTCAG	300
CCTCCCTGGC CATCTCTGGG CTCACGGCTG AGGATGAGGC TGATTATTAC TGCCAGTCCT	360
ATGACAGCAG CCTGAATGGT TATGTCTTCG GAACTGGGAC CCAGCTCACC GTCCTAGGTC	420
AGCCCAAGGC TGCCCCCTCG GTCACCTCTGT TCCCGCCCTC CTCTGAGGAG CTTCAGCCA	480
ACAAGGCCAC ACTGGTGTGT CTCATAAGTG ACTTCTACCC GGGAGCCGTG ACAGTGGCCT	540
GGAAGGCAAT TAGCAGCCCC GTCAAGGCGG GAGTGGAGAC CACCACACCC TCCAACAAAA	600
GCAACAACAA GTACGCGGCC AGCAGCTATC TGAGCCTGAC GCCTGAGCAG TGAAGTCCC	660
ACAGAAGGTA CAGCTGCCAG GTCACGCATG AAGGGAGCAC CGTGGAGAAG ACAGTGGCCC	720
CTACAGAATG TTCATAGTTC TAGATCTACG TATGATCAGC CT	762

## (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Glu Val Gln Leu Leu Glu  
 1 5

## (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 6 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Glu Val Gln Leu Val Glu  
 1 5

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1899 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 14..1735

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGGCAAATA ACA ATG GAG TTG CTA ATC CTC AAA GCA AAT GCA ATT ACC	49
Met Glu Leu Leu Ile Leu Lys Ala Asn Ala Ile Thr	
1 5 10	
ACA ATC CTC ACT GCA GTC ACA TTT TGT TTT GCT TCT GGT CAA AAC ATC	97
Thr Ile Leu Thr Ala Val Thr Phe Cys Phe Ala Ser Gly Gln Asn Ile	
15 20 25	
ACT GAA GAA TTT TAT CAA TCA ACA TGC AGT GCA GTT AGC AAA GGC TAT	145
Thr Glu Glu Phe Tyr Gln Ser Thr Cys Ser Ala Val Ser Lys Gly Tyr	
30 35 40	
CTT AGT GCT CTG AGA ACT GGT TGG TAT ACC AGT GTT ATA ACT ATA GAA	193
Leu Ser Ala Leu Arg Thr Gly Trp Tyr Thr Ser Val Ile Thr Ile Glu	
45 50 55 60	
TTA AGT AAT ATC AAG GAA AAT AAG TGT AAT GGA ACA GAT GCT AAG GTA	241
Leu Ser Asn Ile Lys Glu Asn Lys Cys Asn Gly Thr Asp Ala Lys Val	
65 70 75	
AAA TTG ATA AAA CAA GAA TTA GAT AAA TAT AAA AAT GCT GTA ACA GAA	289
Lys Leu Ile Lys Gln Glu Leu Asp Lys Tyr Lys Asn Ala Val Thr Glu	
80 85 90	
TTG CAG TTG CTC ATG CAA AGC ACA CCA ACA AAC AAT CGA GCC AGA	337
Leu Gln Leu Leu Met Gln Ser Thr Pro Pro Thr Asn Asn Arg Ala Arg	
95 100 105	

AGA GAA CTA CCA AGG TTT ATG AAT TAT ACA CTC AAC AAT GCC AAA AAA	385
Arg Glu Leu Pro Arg Phe Met Asn Tyr Thr Leu Asn Ala Lys Lys	
110 115 120	
ACC AAT GTA ACA TTA AGC AAG AAA AGG AAA AGA AGA TTT CTT GGT TTT	433
Thr Asn Val Thr Leu Ser Lys Lys Arg Lys Arg Phe Leu Gly Phe	
125 130 135 140	
TTG TTA GGT GTT GGA TCT GCA ATC GCC AGT GGC GTT GCT GTA TCT AAG	481
Leu Leu Gly Val Gly Ser Ala Ile Ala Ser Gly Val Ala Val Ser Lys	
145 150 155	
GTC CTG CAC CTA GAA GGG GAA GTG AAC AAG ATC AAA AGT GCT CTA CTA	529
Val Leu His Leu Glu Gly Glu Val Asn Lys Ile Lys Ser Ala Leu Leu	
160 165 170	
TCC ACA AAC AAG GCT GTA GTC AGC TTA TCA AAT GGA GTT AGT GTC TTA	577
Ser Thr Asn Lys Ala Val Val Ser Leu Ser Asn Gly Val Ser Val Leu	
175 180 185	
ACC AGC AAA GTG TTA GAC CTC AAA AAC TAT ATA GAT AAA CAA TTG TTA	625
Thr Ser Lys Val Leu Asp Leu Lys Asn Tyr Ile Asp Lys Gln Leu Leu	
190 195 200	
CCT ATT GTG AAC AAG CAA AGC TGC AGC ATA TCA AAT ATA GAA ACT GTG	673
Pro Ile Val Asn Lys Gln Ser Cys Ser Ile Ser Asn Ile Glu Thr Val	
205 210 215 220	
ATA GAG TTC CAA CAA AAG AAC AAC AGA CTA CTA GAG ATT ACC AGG GAA	721
Ile Glu Phe Gln Gln Lys Asn Asn Arg Leu Leu Glu Ile Thr Arg Glu	
225 230 235	
TTT AGT GTT AAT GCA GGT GTA ACT ACA CCT GTA AGC ACT TAC ATG TTA	769
Phe Ser Val Asn Ala Gly Val Thr Thr Pro Val Ser Thr Tyr Met Leu	
240 245 250	
ACT AAT AGT GAA TTA TTG TCA TTA ATC AAT GAT ATG CCT ATA ACA AAT	817
Thr Asn Ser Glu Leu Leu Ser Leu Ile Asn Asp Met Pro Ile Thr Asn	
255 260 265	
GAT CAG AAA AAG TTA ATG TCC AAC AAT GTT CAA ATA GTT AGA CAG CAA	865
Asp Gln Lys Lys Leu Met Ser Asn Asn Val Gln Ile Val Arg Gln Gln	
270 275 280	
AGT TAC TCT ATC ATG TCC ATA ATA AAA GAG GAA GTC TTA GCA TAT GTA	913
Ser Tyr Ser Ile Met Ser Ile Ile Lys Glu Glu Val Leu Ala Tyr Val	
285 290 295 300	
GTA CAA TTA CCA CTA TAT GGT GTT ATA GAT ACA CCC TGT TGG AAA CTA	961
Val Gln Leu Pro Leu Tyr Gly Val Ile Asp Thr Pro Cys Trp Lys Leu	
305 310 315	
CAC ACA TCC CCT CTA TGT ACA ACC AAC ACA AAA GAA GGG TCC AAC ATC	1009
His Thr Ser Pro Leu Cys Thr Thr Asn Thr Lys Glu Gly Ser Asn Ile	
320 325 330	
TGT TTA ACA AGA ACT GAC AGA GGA TGG TAC TGT GAC AAT GCA GGA TCA	1057
Cys Leu Thr Arg Thr Asp Arg Gly Trp Tyr Cys Asp Asn Ala Gly Ser	
335 340 345	
GTA TCT TTC TTC CCA CAA GCT GAA ACA TGT AAA GTT CAA TCA AAT CGA	1105
Val Ser Phe Phe Pro Gln Ala Glu Thr Cys Lys Val Gln Ser Asn Arg	
350 355 360	



GTA TTT TGT GAC ACA ATG AAC AGT TTA ACA TTA CCA AGT GAA ATA AAT	1153
Val Phe Cys Asp Thr Met Asn Ser Leu Thr Leu Pro Ser Glu Ile Asn	
365 370 375 380	
CTC TGC AAT GTT GAC ATA TTC AAC CCC AAA TAT GAT TGT AAA ATT ATG	1201
Leu Cys Asn Val Asp Ile Phe Asn Pro Lys Tyr Asp Cys Lys Ile Met	
385 390 395	
ACT TCA AAA ACA GAT GTA AGC AGC TCC GTT ATC ACA TCT CTA GGA GCC	1249
Thr Ser Lys Thr Asp Val Ser Ser Ser Val Ile Thr Ser Leu Gly Ala	
400 405 410	
ATT GTG TCA TGC TAT GGC AAA ACT AAA TGT ACA GCA TCC AAT AAA AAT	1297
Ile Val Ser Cys Tyr Gly Lys Thr Lys Cys Thr Ala Ser Asn Lys Asn	
415 420 425	
CGT GGA ATC ATA AAG ACA TTT TCT AAC GGG TGC GAT TAT GTA TCA AAT	1345
Arg Gly Ile Ile Lys Thr Phe Ser Asn Gly Cys Asp Tyr Val Ser Asn	
430 435 440	
AAA GGG ATG GAC ACT GTG TCT GTA GGT AAC ACA TTA TAT TAT GTA AAT	1393
Lys Gly Met Asp Thr Val Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn	
445 450 455 460	
AAG CAA GAA GGT AAA AGT CTC TAT GTA AAA GGT GAA CCA ATA ATA AAT	1441
Lys Gln Glu Gly Lys Ser Leu Tyr Val Lys Gly Glu Pro Ile Ile Asn	
465 470 475	
TTC TAT GAC CCA TTA GTA TTC CCC TCT GAT GAA TTT GAT GCA TCA ATA	1489
Phe Tyr Asp Pro Leu Val Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile	
480 485	
TCT CAA GTC AAC GAG AAG ATT AAC CAG AGC CTA GCA TTT ATT CGT AAA	1537
Ser Gln Val Asn Glu Lys Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys	
495 500 505	
TCC GAT GAA TTA TTA CAT AAT GTA AAT GCT GGT AAA TCC ACC ACA AAT	1585
Ser Asp Glu Leu Leu His Asn Val Asn Ala Gly Lys Ser Thr Thr Asn	
510 515 520	
ATC ATG ATA ACT ACT ATA ATT ATA GTG ATT ATA GTA ATA TTG TTA TCA	1633
Ile Met Ile Thr Thr Ile Ile Ile Val Ile Ile Val Ile Leu Leu Ser	
525 530 535 540	
TTA ATT GCT GTT GGA CTG CTC TTA TAC TGT AAG GCC AGA AGC ACA CCA	1681
Leu Ile Ala Val Gly Leu Leu Leu Tyr Cys Lys Ala Arg Ser Thr Pro	
545 550 555	
GTC ACA CTA AGC AAA GAT CAA CTG AGT GGT ATA AAT AAT ATT GCA TTT	1729
Val Thr Leu Ser Lys Asp Gln Leu Ser Gly Ile Asn Asn Ile Ala Phe	
560 565 570	
AGT AAC TAAATAAAAA TAGCACCTAA TCATGTTCTT ACAATGGTTT ACTATCTGCT	1785
Ser Asn	
CATAGACAAC CCATCTGTCA TTGGATTTTC TTAAATCTG AACTTCATCG AAATCTCAT	1845
CTATAAACCA TCTCACTTAC ACTATTAAAG TAGATTCCCTA GTTTATAGTT ATAT	1899

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 574 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

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Met Glu Leu Leu Ile Leu Lys Ala Asn Ala Ile Thr Thr Ile Leu Thr
 1           5           10           15

Ala Val Thr Phe Cys Phe Ala Ser Gly Gln Asn Ile Thr Glu Phe
          20           25           30

Tyr Gln Ser Thr Cys Ser Ala Val Ser Lys Gly Tyr Leu Ser Ala Leu
          35           40           45

Arg Thr Gly Trp Tyr Thr Ser Val Ile Thr Ile Glu Leu Ser Asn Ile
          50           55           60

Lys Glu Asn Lys Cys Asn Gly Thr Asp Ala Lys Val Lys Leu Ile Lys
          65           70           75           80

Gln Glu Leu Asp Lys Tyr Lys Asn Ala Val Thr Glu Leu Gln Leu Leu
          85           90           95

Met Gln Ser Thr Pro Pro Thr Asn Asn Arg Ala Arg Arg Glu Leu Pro
          100          105          110

Arg Phe Met Asn Tyr Thr Leu Asn Asn Ala Lys Lys Thr Asn Val Thr
          115          120          125

Leu Ser Lys Lys Arg Lys Arg Arg Phe Leu Gly Phe Leu Leu Gly Val
          130          135          140

Gly Ser Ala Ile Ala Ser Gly Val Ala Val Ser Lys Val Leu His Leu
          145          150          155          160

Glu Gly Glu Val Asn Lys Ile Lys Ser Ala Leu Leu Ser Thr Asn Lys
          165          170          175

Ala Val Val Ser Leu Ser Asn Gly Val Ser Val Leu Thr Ser Lys Val
          180          185          190

Leu Asp Leu Lys Asn Tyr Ile Asp Lys Gln Leu Leu Pro Ile Val Asn
          195          200          205

Lys Gln Ser Cys Ser Ile Ser Asn Ile Glu Thr Val Ile Glu Phe Gln
          210          215          220

Gln Lys Asn Asn Arg Leu Leu Glu Ile Thr Arg Glu Phe Ser Val Asn
          225          230          235          240

Ala Gly Val Thr Thr Pro Val Ser Thr Tyr Met Leu Thr Asn Ser Glu
          245          250          255

Leu Leu Ser Leu Ile Asn Asp Met Pro Ile Thr Asn Asp Gln Lys Lys
          260          265          270

Leu Met Ser Asn Asn Val Gln Ile Val Arg Gln Gln Ser Tyr Ser Ile
          275          280          285

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Met Ser Ile Ile Lys Glu Glu Val Leu Ala Tyr Val Val Gln Leu Pro
 290                               295                               300

Leu Tyr Gly Val Ile Asp Thr Pro Cys Trp Lys Leu His Thr Ser Pro
 305                               310                               315

Leu Cys Thr Thr Asn Thr Lys Glu Gly Ser Asn Ile Cys Leu Thr Arg
                               325                               330                               335

Thr Asp Arg Gly Trp Tyr Cys Asp Asn Ala Gly Ser Val Ser Phe Phe
 340                               345                               350

Pro Gln Ala Glu Thr Cys Lys Val Gln Ser Asn Arg Val Phe Cys Asp
 355                               360                               365

Thr Met Asn Ser Leu Thr Leu Pro Ser Glu Ile Asn Leu Cys Asn Val
 370                               375                               380

Asp Ile Phe Asn Pro Lys Tyr Asp Cys Lys Ile Met Thr Ser Lys Thr
 385                               390                               395

Asp Val Ser Ser Ser Val Ile Thr Ser Leu Gly Ala Ile Val Ser Cys
                               405                               410                               415

Tyr Gly Lys Thr Lys Cys Thr Ala Ser Asn Lys Asn Arg Gly Ile Ile
                               420                               425                               430

Lys Thr Phe Ser Asn Gly Cys Asp Tyr Val Ser Asn Lys Gly Met Asp
 435                               440                               445

Thr Val Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn Lys Gln Glu Gly
 450                               455                               460

Lys Ser Leu Tyr Val Lys Gly Glu Pro Ile Ile Asn Phe Tyr Asp Pro
 465                               470                               475                               480

Leu Val Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile Ser Gln Val Asn
 485                               490                               495

Glu Lys Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys Ser Asp Glu Leu
 500                               505                               510

Leu His Asn Val Asn Ala Gly Lys Ser Thr Thr Asn Ile Met Ile Thr
 515                               520                               525

Thr Ile Ile Ile Val Ile Ile Val Ile Leu Leu Ser Leu Ile Ala Val
 530                               535                               540

Gly Leu Leu Leu Tyr Cys Lys Ala Arg Ser Thr Pro Val Thr Leu Ser
 545                               550                               555                               560

Lys Asp Gln Leu Ser Gly Ile Asn Asn Ile Ala Phe Ser Asn
 565                               570

```

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
1				5					10					15

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/13694

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/395, 39/42; C12Q 1/00, 1/70; G01N 33/53  
US CL : 424/130.1, 141.1, 147.1; 435/4, 5, 7.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 141.1, 147.1; 435/4, 5, 7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 5,811,524 A (BRAMS et al) 22 September 1998, cols. 12-20.	1, 4, 10-15 ----- 2, 3
X ----- Y	US 5,824,307 A (JOHNSON) 20 October 1998, cols. 4-6.	1, 4, 10-15 ----- 2, 3
X ----- Y	US 5,880,104 A (LI et al) 09 March 1999, cols. 6-10.	1, 4, 10-15 ----- 2, 3

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

\* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*B\* earlier document published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

\*Z\* document member of the same patent family

Date of the actual completion of the international search

16 AUGUST 2000

Date of mailing of the international search report

05 SEP 2000

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/13694

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, DIALOG, MEDLINE

search terms: RSV, respiratory syncytial, monoclonal, antibodies, human, humanized, F protein, diagnostics, passive immunization, therapy, treatment